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Compiled by

Ranzell Nickelson II

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TABLE OF CONTENTS

MODIFIED DILUTION PROCEDURE FOR BACTERIOLOGICAL EXAMINATION OF SEAFOODS

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INTRODUCTION

The APHA publication entitled "Recommended Procedures for the Examination of Sea Water and Shellfish" (1) is the manual followed by all state and federal regulatory laboratories in the examination of shellfish for bacteria. The procedure described therein specifies that approximately 200g of shucked shellfish be homogenized with an equal weight of diluent. Following a 60 to 120 second grinding time in a laboratory blender, the mixture is ready for dilution and inoculation into culture media. The procedure recommends that the ground sample be cultured within 2 minutes after the grinding period is completed.

It has been our experience when working with oysters that the homogenate is quite frothy; a result of air being whipped into the mixture during blending. The frothiness of the homogenate appears to depend on the physiological condition of the oysters, the post harvest age of the oysters, and mechanical factors of the blending process including style of blender cup, blending speed and time. Standing time required for separation of the liquid and air exceeds 30 minutes.

The APHA specifies that results of bacteriological analysis on shellfish be expressed on a weight basis and all bacteriological standards for shellfish are expressed on a weight basis. However, steps in the analysis after the initial blending are carried out on a volume basis. Therefore any steps in the analytical procedure which may significantly alter the volume to weight ratio in the sample will effect the accuracy of bacteriological results obtained from those samples.

It was the purpose of this study to document the fact that the APHA procedure underestimates the number of bacteria in oyster samples because it does not correct for changes in the volume of the sample during homogenization and to recommend a change to correct inadequacies of the procedure.

METHODS

Oyster samples were obtained from processing plants or retail outlets as either shellstock or shucked samples. Winter samples were those obtained in January through March and the summer samples April through September.

APHA procedures (1) were followed throughout the study except where indicated otherwise.

The modified procedure for oyster homogenate dilution and inoculation into presumptive medium is shown in figure 1 and differs from the APHA procedure in three points: (a) preparation of the first dilution of the homogenate on a weight basis rather than a volume basis. This is accomplished by pipetting homogenate into a tared dilution bottle containing 80 ml of diluent until 20g of homogenate has been added. (b) inoculation of the first series of tubes of presumptive medium with the dilution prepared on a weight basis rather than inoculation of the homogenate directly into the presumptive medium and (c) use of double strength presumptive medium to receive the 1g portions of sample.

In the paired fecal coliform analysis, two technicians were involved so that both procedures for dilution and inoculation could be carried out on each sample simultaneously and immediately after oyster homogenization. The technicians alternated procedures on each sample to reduce technical bias in the results.

RESULTS AND DISCUSSION

To test our hypothesis that the shellfish homogenization procedure as described by APHA changed the volume to weight ratio in the sample, we measured the volume of homogenate required to equal 20g. This weight was selected bacause the first step in the APHA dilution procedure prescribes that 20 ml of shellfish homogenate be added to 80 ml of dilution water and states that each ml of this dilution will represent 0.1g, thus inferring that 20 ml of the homogenate equals 20g. Data in table 1 indicates a wide range in volume of homogenate required to equal 20g, and that the volume varied with the time of year and the source of sample. Oysters harvested during the winter have a higher glycogen content and upon blending produce a thicker homogenate which entraps more air. Plant shucked samples had less shell liquor than did samples of shellstock shucked in the lab.

Table 1. Volume of oyster homogenate required to equal 20g and error caused by assuming 20 ml of homogenate weighs 20g

Source	Number of Samples	ml equal to 20g Range	ml equal to 20g Mean	Mean % Error
Winter Samples	46	21.5-28	24.7	23.5
Summer Samples	41	20.5-26	23.3	16.5
Shellstock Samples	16	20.5-26	23.7	18.5
Plant Shucked Samples	71	21.5-28	24.2	21.0
Overall	87	20.5-28	24.1	20.5

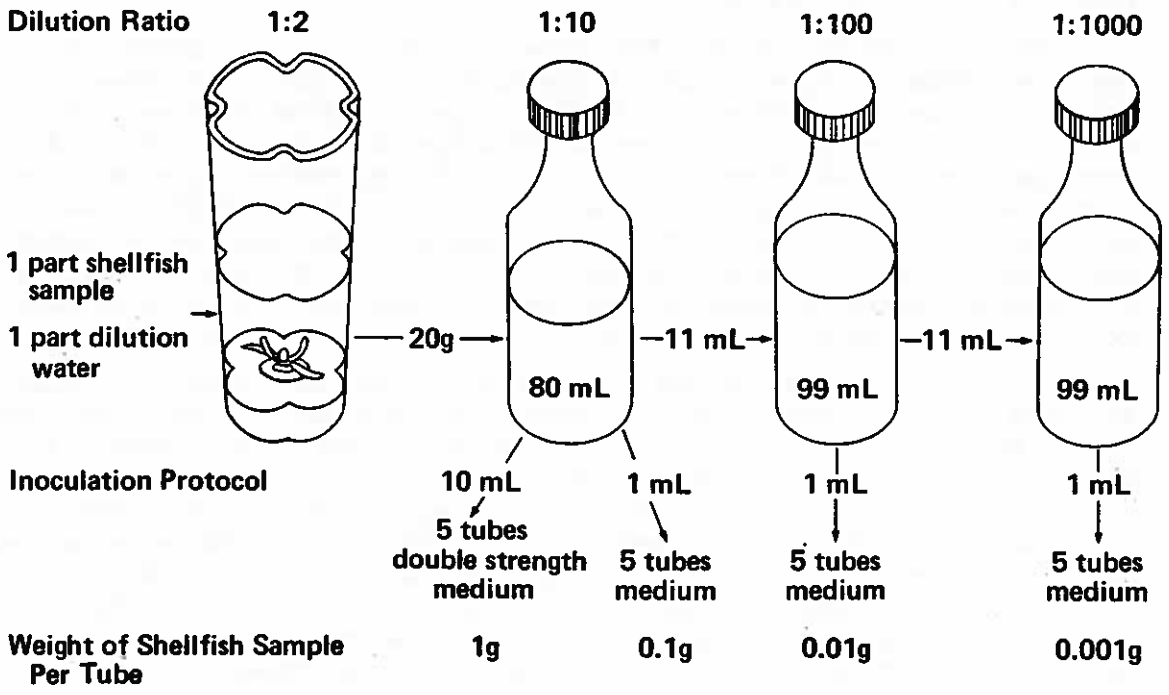

Figure 1. Modified dilution and inoculation procedure for analysis of fecal coliforms in shellfish.

In the analysis of oyster samples for fecal coliform, the accuracy is further compromised because of the difficulty in accurately pipetting the two ml sample (supposedly equal to 1.0 g of oyster tissue) of the thick and often clumpy homogenate into the culture tube. We suggest a modification of the APHA procedure as outlined in the METHODS section to overcome the inadequacies of that procedure.

To test if the modified procedure produced a difference in bacteriological counts as predicted by the volume to weight difference, fecal coliform analyses were performed of 49 oyster samples using both procedures. The volume of homogenate required to equal 20 g ranged from 21.5 to 28.0 ml ($\bar{x}$ = 24.7 ml). Error resulting from preparation of the first dilution by volume rather than weight could be expected to range from 7.5 to 40% ($\bar{x}$ = 23.5%).

Since the fecal coliform MPN values are based on the number of positive tubes, we first compared the number of positive EC-tubes produced by each procedure. In 40.8% of the 49 paired analyses, the modified procedure produced more positive EC-tubes than did the APHA procedure, and in 44.9% of the analyses, both procedures gave the same number of positive tubes. A paired t-test (t = 2.640, 48 df) revealed that the number of positive EC-tubes produced by the modified procedure was significantly increased compared with the APHA procedure. Overall, the APHA procedure recorded 89 positive EC-tubes whereas the modified procedure gave 110 positive tubes, or an increase of 23.6%.

Fecal coliform MPN values were computed from the positive EC-tube data and further comparisons were made. The modified procedure gave MPN values which were higher than those obtained by the APHA procedure in 42.9% of the paired analyses. In 26.5% of the paired analyses, both procedures produced equal MPN data. A paired t-test was performed on log-transformed MPN data and indeterminate MPN values (<18) were assigned an arbitrary value of 1. This test showed there was a significant difference in the two procedures (t = 2.067, 48 df). When the MPN values from each procedure were ranked separately, the median values for the modified and APHA procedures were 40 and 20, respectively.

Aerobic plate counts were performed on 14 of these oyster samples. One ml portions of the 1:10, 1:100 and 1:1000 dilutions prepared by both procedures were used to inoculate plate count agar. In 71.4% of the paired analyses, the modified procedure gave higher counts than did the APHA procedure.

These data indicate that the modified procedure recovers a significantly greater number of bacteria from oyster samples and that this difference is traceable to the fact that the APHA procedure does not account for errors produced when air is introduced into the homogenate during blending. This difficulty can be overcome by adopting the dilution procedure described herein which calls for the initial dilution of the oyster homogenate to be made on a weight rather than a volume basis. There is no reason to believe that other species of molluscan shellfish would produce results different from those obtained with oysters.

There is no separate protocol for the microbiological analysis of seafoods other than the molluscan shellfish. Shrimp and crabmeat are usually analyzed using procedures recommended for solid or semi-solid foods by FDA (2), AOAC (4) or APHA (7). These procedures involve homogenizing 50 grams of the food with 450 ml of dilution water for 2 minutes. They further recommend that all subsequent dilutions be made on a volume basis and that all inoculations be completed within 15 minutes.

Shrimp homogenates are quite foamy immediately after blending. Considerable error (10 to 40%, $\bar{x}$ = 20.6%) may result if dilutions of the foamy homogenate are prepared on a volume basis immediately after blending. Several researchers (3, 5) have recommended allowing the homogenates to rest for 2 to 5 minutes before pipetting which allows most of the air to excape from the mixture. However, since shrimp in different forms (raw headless, peeled, cooked, etc.) respond differently during homoginization and their foams separate at different rates, we recommend, as others (6) have, that the first dilution of the homogenate be prepared on a weight rather than a volume basis.

CONCLUSION

As shown in this study, considerable error can be introduced into an analytical procedure by a change in the density of the sample during homoginization in a blender. Persons involved in bacteriological analysis of seafoods should be aware of this problem and we recommend that the first dilution of all homogenates be prepared on a weight basis to eliminate the error.

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OCCURRENCE AND DISTRIBUTION OF *Salmonella* IN THE SUWANNEE RIVER ESTUARY

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INTRODUCTION

In previous reports from this laboratory, data was presented on the recovery of salmonellae from seafood harvested within the Suwannee River estuary (4,5). The isolation rate of salmonellae from these products was surprisingly high and therefore raised the question as to the source of these organisms. Clams, oysters and crabs were all found to contain *Salmonella*, ranging from 12 to 35% of the samples analyzed, depending upon the species.

The Suwannee River estuary is a vast relatively undeveloped area receiving approximately 7 billion gallons of water per day. The river flows south west out of the Okefenokee Swamp in Georgia and empties into the Gulf of Mexico at Suwannee, FL. Pollution is mainly limited to that occurring from phosphate mining and from poorly operating septic tanks scattered throughout the area. Development has been curtailed because of the lack of available high ground on which to build. Further restrictions to development are being enacted by the 11 counties in Florida through which the river flows.

In order to better understand this salmonellae problem, ten stations were established within the estuary and monitored for the occurrence of salmonellae using Moore swabs. In addition, the marsh clam, a biological monitor, was sampled from a location at the mouth of the river and analyzed for salmonellae every three months. Total and fecal coliform analyses were also conducted on water samples collected at the time the Moore swabs were removed from the water. It was believed that these two systems would better indicate the status of the area than just a single system alone and would yield information regarding the occurrence and distribution of *Salmonella* in the estuary throughout the year.

MATERIALS AND METHODS

Sites: Ten sites in the Suwannee River estuary were selected for sampling (Fig. 1). Sites 1, 2, 3 and 10 are located at the mouth of the two passes opening into the Gulf, while sites 4, 6, 7, 8 and 9 are located in the river. Site 5 is within a housing development and is in a dead end canal. From site 1 to 8 is aproximately 7000 meters.

Samples: Moore swabs (13) were prepared so that when saturated with water they would weigh approximately 40 g. Each swab consisted of 15 strips of cotton cheesecloth (20 x 4 cm) tied in a bundle at the top leaving the ends free. Two swabs were attached to a nylon rope and anchored at each station. The swabs were positioned about 50 cm below the surface of the

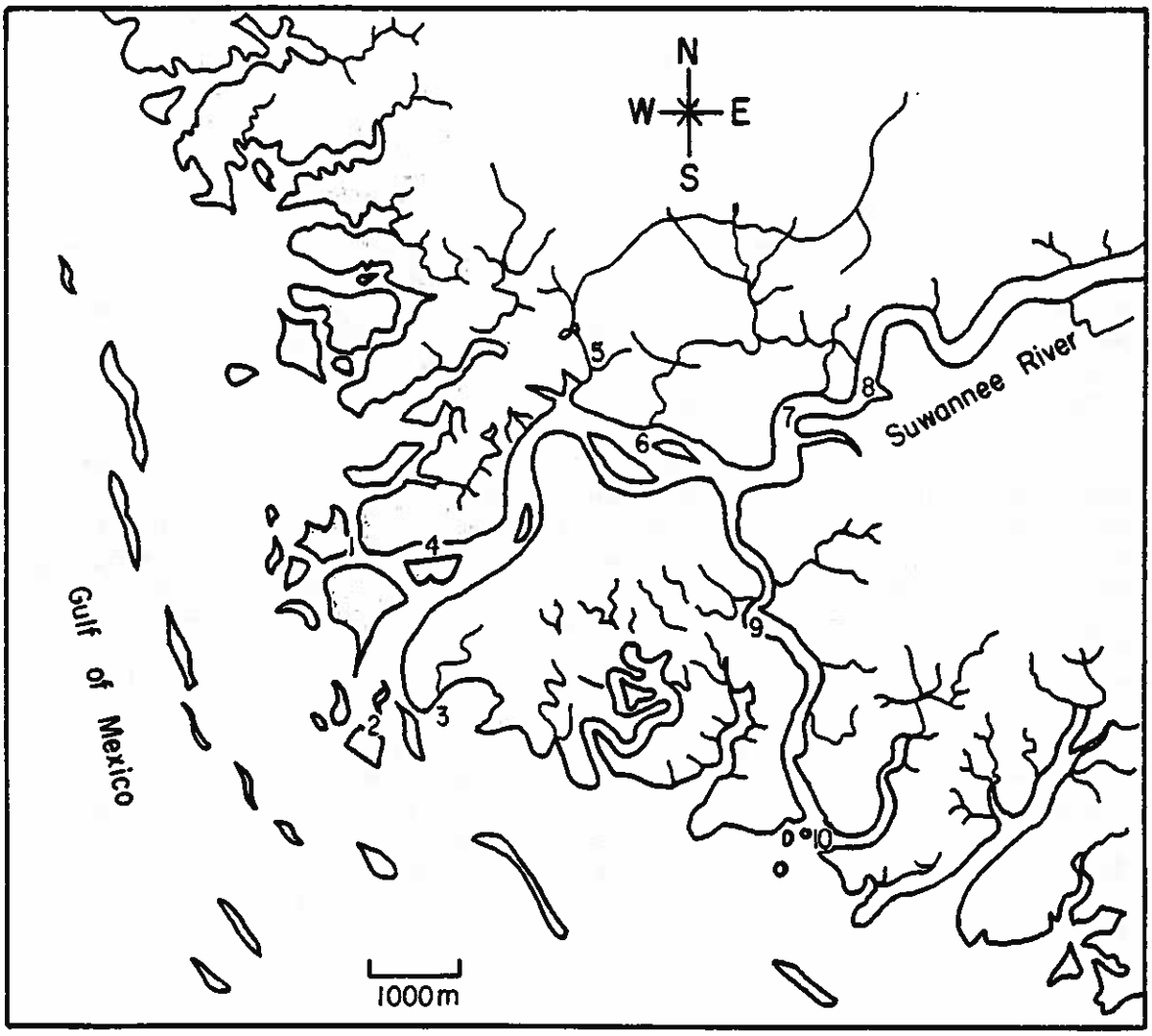

FIG. 1. Map of Suwannee River estuary showing collection stations.

water by a float at the end of the rope. Soak time for the swabs was 3 days. At the time of collection, the swabs were placed in sterile plastic bags and returned to the laboratory. Also at the time of collection, a water sample was obtained at each site to be analyzed for total and fecal coliforms, and salinity. The temperature of the water was also taken at each site.

Marsh clams (Polymesoda caroliniana) were collected at low tide from a mud flat located at station 2, placed in sterile plastic bags and transported to the laboratory in Gainesville, FL for analysis.

Analyses: For Salmonella analysis of the Moore swabs, each swab was weighed and a 1:10 dilution made using Selenite Cystine broth. The samples were incubated at 42C for 24 h, then streaked onto Xylose Lysine Desoxycholate, Bismuth Sulfite and Brilliant Green agars. The plates were incubated at 35C for 24 h, and typical colonies were transferred to Triple Sugar Iron and Lysine Iron agar slants. Presumptive Salmonella isolates were analyzed biochemically using standard procedures (12,15) followed by serological testing using Polyvalent O antisera.

Salmonella analysis of the marsh clams was performed in the same manner as the Moore swabs except that a 10 g MPN procedure was run in duplicate on the clams. Approximately 4 to 5 clams were blended at 8000 rpm in a Hamilton Beach blender for each sample. Ten gram portions were transferred to each of five 90-ml bottles of Selenite Cystine per sample. The number of Salmonella-positive bottles were then determined by the same procedures as above to obtain an MPN value (10).

In addition to the serological testing done in our lab, specific serotyping of the October Moore swab and the November marsh clam salmonelleae isolates was done by the General Bacteriology Laboratory of the Department of Health and Rehabilitative Services in Jacksonville, FL.

Coliform analyses on the marsh clam and water samples were done by the standard 5 tube MPN procedures (12,15). Salinity was determined using a hydrometer to determine specific gravity as described by Pickard (11).

$$\text{Salinity (0/00)} = (\text{specific gravity} - 1) \times 1{,}323$$

A correlation analysis was also run between the MPN of Salmonella present in the marsh clams and water temperature.

RESULTS AND DISCUSSION

Various workers have raised questions regarding the occurrence, distribution, survival and growth of a number of organisms of public health significance in the aquatic environment (1,3,6,8,14). These studies have ranged from growth of Escherichia coli in the Mississippi River to the recovery of Salmonella from mountain top pools in Georgia. These studies and their interpretation are frustrated by the usual technical difficulties associated with research conducted in the field. However, all have raised the question regarding the ability of these organisms to survive and grow under natural conditions.

Our data clearly points out the wide distribution of salmonellae in the Suwannee River estuary (Table 1). All sampling dates, except for the February clam samples, yielded positive samples for Salmonella. Our failure to recover salmonellae from these samples was attributed to the low temperature of the water at that time and therefore the lack of feeding activity by the clams. This assumption is supported by the fact that during this same period all Moore swabs were positive for Salmonella.

Table 1. Recovery of Salmonella from samples obtained in the Suwannee River estuary.

	Marsh Clams			
	MPN/g[b]			Moore Swabs[a]
Month	Total Coliforms	Fecal Coliforms	Salmonella	Number positive for Salmonella/Number analyzed
Aug. '82	731	28	0.16	
Oct.				20/20
Nov.	94	20	0.05	
Feb. '83	46	< 2	0	20/20
May	33	< 2	0.01	
Jun.				18/18[c]
Aug.	391	17	> 0.16	

[a]Two swabs analyzed for Salmonella from each of 10 sampling stations.

[b]Values represent the average of duplicate samples.

[c]Swabs from station 3 were lost.

Correlation analysis done using the MPN/g of Salmonella present in marsh clam samples as related to the water temperature showed a very high correlation value, R^2=0.94 (Table 2). Using 99% confidence, it was determined that there is a direct relationship between these two variables; the MPN value for Salmonella increased as the temperature of the water increased.

Serotyping of the isolates from the October Moore swabs yielded 13 different serotypes present in the water. There were 3 serotypes isolated from the November clam samples (Table 3).

The coliform analyses conducted on the water samples from the 10 stations were high based on Federal water quality standards (Table 4). However, from a more pragmatic view, shellfish harvested from areas receiving large quantities of fresh water, as does the Suwannee area, historically have difficulty meeting these standards. It appears that the continuous wash out of organisms from the soil into the water, as well as their continual growth, is responsible for some of the coliform contamination in the water. This assumption is

Table 2. Marsh Clams: Correlation analysis of *Salmonella* present to water temperature.

Month	Salmonella (MPN/g)	Water Temperature (°C)
Aug. '82	0.16	28.0
Nov.	0.05	17.8
Feb. '83	0	11.0
May	0.01	16.5
Aug.	> 0.16	26.6

Correlation coefficient: R^2=0.94.

Table 3. *Salmonella* serotypes isolated from Moore swabs and Marsh clams.

October Moore Swabs		November Marsh Clams
S. allandale	*S. montevideo*	*S. allandale*
S. bareilly	*S. muenchen*	*S. hartford*
S. berta	*S. newport*	*S. inverness*
S. hartford	*S. oranienburg*	
S. heidelberg	*S. saint paul*	
S. inverness	*S. tallahassee*	
S. java		

Table 4. Water coliforms and Moore swab Salmonella data from 10 stations in the Suwannee River estuary.

Station	Total Coliforms[a]			Fecal Coliforms[a]			Salmonella		
	Oct.	Feb.	Jun.	Oct.	Feb.	Jun.	Oct.	Feb.	Jun.
1	3.3	3.1	7.0	< 0.2	0.5	0.8	+[c]	+	+
2	13.0	1.3	8.0	0.2	0.8	0.7	+	+	+
3	7.9	2.2	Nd[b]	0.7	1.1	Nd	+	+	Nd
4	10.9	1.7	1.1	0.4	0.8	0.2	+	+	+
5	13.0	3.3	Nd	0.5	1.1	Nd	+	+	+
6	2.2	3.3	4.6	0.2	0.8	0.2	+	+	+
7	3.3	1.3	1.1	0.8	0.8	0.5	+	+	+
8	4.9	1.7	0.8	0.2	0.5	< 0.2	+	+	+
9	10.9	4.6	4.9	0.5	1.1	0.2	+	+	+
10	3.3	3.1	3.3	0.5	1.3	0.5	+	+	+

[a] MPN/g.

[b] No data.

[c] Both swabs positive for Salmonella.

based on data collected at station 5. Station 5 is a dead end canal located within a housing development serviced by septic tanks, and the coliform counts are not markedly different from the other stations, some far removed from major sources of obvious contamination. Other possibilities exist that might account for the presence of salmonellae such as dissemination by birds and animals; however, these options are extremely difficult to prove. In addition, salinities and water temperatures were not extreme, thereby not inhibiting growth of these organisms (Table 5).

Florida is unique among the states in that it experiences a relatively mild winter. The lowest water temperature recorded during this study was 11C during the February sampling period. This is a temperature above the lowest stated temperature for growth of salmonellae. This factor in addition to an availability of nutrients and tidal movement probably accounts for the growth and dissemination of these organisms under these conditions.

Previous reports have documented or alluded to the ability of a number of entero-pathogens to survive and grow removed from a host support system (2,3,7,9). These have included such organisms as Escherichia coli, Vibrio cholerae as well as Salmonella. V. cholerae is the most recent addition to this list, with acceptance of its autochthonous status. Therefore, it is not unreasonable to hypothesize that a similar situation is possible with the salmonellae, thereby accounting for the level of contamination found in the water and various seafoods harvested from this and similar areas.

Table 5. Salinity and temperature determinations of water from the 10 stations in the Suwannee River estuary.

Station	Salinity (0/100)			Temperature (C)		
	Oct.	Feb.	Jun.	Oct.	Feb.	Jun.
1	1.323	0.132	0	17.0	12.0	24.8
2	0.926	0.662	0	17.5	12.0	24.4
3	Nd[a]	0.132	Nd	15.0	12.0	24.0
4	0	0	0	18.1	12.0	24.6
5	0	0	Nd	17.8	12.8	25.8
6	0	0	0	18.4	12.0	23.0
7	0	0	0	18.8	12.1	23.0
8	0	0	0	18.5	12.0	22.8
9	0	0	0	18.3	12.0	23.5
10	0.662	0	0	18.0	12.1	24.8

[a]No data.

ACKNOWLEDGEMENTS

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DETERMINATION OF THE THERMAL DEATH TIME OF *VIBRIO CHOLERAE* IN CRAYFISH MEAT HOMOGENATE (*PROCAMBARUS CLARKII GERARD*)

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INTRODUCTION

The consumption of crayfish has been a custom in southern Louisiana for over 2 centuries. Today, Louisiana is responsible for the growth, processing, and consumption of 90% of the crayfish produced for human consumption in the United States (3), but recently other states and countries have expressed an interest in the crayfish.

Crayfish are harvested from bayous, swamps, lakes, and man-made crayfish ponds (3). Crayfish may possibly harbor pathogenic microorganisms if they are taken from contaminated waters. In the past, fecal microorganisms have been isolated from crayfish waters, whole crayfish, and crayfish tailmeat (1).

Crabs taken from contaminated waters were responsible for 11 *Vibrio cholerae* infections in Louisiana in 1978 (2). Although the crabs were reportedly boiled or steamed, the cooking was not sufficient to destroy all *V. cholerae* present or the cooked crabs were recontaminated with the organism before consumption.

The purpose of this study was to determine the D values of *V. cholerae* 01 in crayfish tailmeat, to determine the effect of two methods of cooking on *V. cholerae* injected into whole crawfish, and to analyze the rate of heat penetration in the whole crayfish while cooking.

MATERIALS AND METHODS

Organism. *Vibrio cholerae* 01, biotype El Tor, serotype Inaba (Louisiana strain #5875) was obtained from the Department of Microbiology at Louisiana State University. The organism was stored at room temperature on 1% peptone-salt agar slants and transferred to fresh media at monthly intervals.

Preparation and inoculation of the crayfish homogenate. Packaged crayfish tail meat was purchased from local seafood stores and stored at -18C until ready for use. Approximately 3 parts thawed crayfish meat and 1 part distilled water were blended at high speed in a Waring blendor until smooth.

Vibrio cholerae grown in nutrient broth for 20 to 24 hr at 35C was added to the homogenate to yield a final concentration of

approximately 10^6 V. cholerae/g (5). The inoculated homogenate was throughly mixed by blending at high speed for 2 min.

Preparation of thermal-death-time tubes. Pyrex glass tubing with an outer diameter of 13 mm was cut into 15 cm lengths and heat sealed at one end with an oxygen-acetylene torch.

Four grams of the crayfish homogenate were placed into the tubes with a sterile animal force feeder. This resulted in 4.0×10^6 V. cholerae/tube or a total of 6.0×10^7 V. cholerae in the 15 thermal death-time (TDT) tubes. The tubes were heated sealed in 10 cm lengths.

Determination of the come-up time. The come-up time (time required for the temperature of the samples to rise from room temperature to the treatment temperature) was determined at each temperature. Six of the 15 TDT tubes were equipped with thermocouples connected to a Leeds and Northrup Speedomax multipoint recorder. The time required for the slowest heating tube to reach the treatment temperature was used as the come-up time for all other experiments at that temperature. The timing of heat treatments was begun at the end of the come-up period. The lethal effect of the heat during the come-up time was determined mathematically (7).

Endpoint determination. The tubes containing the crayfish homogenate were totally submerged in a water bath for various time increments at 66, 71, 77, and 82C respectively. Following heating, the tubes were cooled in an ice-water bath for 30 seconds and V. cholerae if present was recovered using an alkaline peptone water (APW) enrichment and isolation on thiolsulfate-citrate-bile salts-sucrose (TCBS; Difco) and gelatin agar (8). The contents of the TDT tubes were aseptically transferred to tubes containing APW. The APW tubes were incubated at 35C for 6 to 8 hr. Contents from each tube of APW were plated on TCBS and gelatin agar plates. After incubation at 35C for 20 to 24 hr suspect colonies for each plate were transferred to a three tube series of Klieger's iron agar (KIA; Difco), lysine iron agar (LIA; Difco), and peptone-slant agar (PSA). Cultures producing positive reactions on KIA and LIA were checked by serological identification procedures. Organisms taken from the PSA slants (from positive series) were tested for agglutination on Bacto Vibrio cholerae Polyvalent and Inaba antiserum (Difco). Recovery of V. cholerae from the heated homogenates was recorded as "growth" or "no growth" at each time-temperature combination.

The control sample consisted of inoculated homogenate which underwent the same treatment as the experimental sample, except no heat treatment was applied.

Heat penetration. Commercially harvested crayfish were purchased from local seafood retailers and stored at 4C until ready for use.

The internal temperature of the crayfish during cooking in water (100C) and in steam (100C) was measured. Two 20 gauge,

Teflon-insulated, copper-constantan duplex wire thermocouples were inserted into each crayfish. One was inserted in the thickest portion of the tail muscle and the other in the central body cavity of the cephalothorax region. Twenty-four crayfish were used for each cooking method, and the weight of each crayfish was recorded to the nearest gram. The crayfish were cooked in a Dixie Retort (Model RDT13) equipped with pneumatic instrumentation suitable for processing in a water or steam cook. For steaming at atmospheric pressure, timing started once the mercury-in-glass thermometer indicated 100C inside the closed retort. When processing in water, timing started once the crayfish were placed in the boiling solution. Temperatures were recorded over a 10 min period on the 12 point strip chart recorder.

Inoculated packs: Live crayfish were injected with 1 ml of saline containing 10^6 V. cholerae. Six crayfish were injected in the tail muscle and 6 were injected in the central body cavity of the cephalothorax region. Three of the crayfish which had been injected by either method were cooked in 100C water or 100C steam for 5 minutes. Vibrio cholerae if present was recovered from the cooked crayfish by blending the crayfish in a 9 part APW media and incubating for 6 to 8 hr at 35C. The V. cholerae was then isolated and identified as described above. A control sample consisting of inoculated crayfish was treated in the same manner as above, except that the crayfish were not cooked.

RESULTS AND DISCUSSION

D values. The endpoint at each temperature was based on the heating period after which no V. cholerae were recovered from the 15 TDT tubes containing a total of 6.0×10^7 V. cholerae.

Vibrio cholerae was not recovered from any of the 15 tubes which contained the inoculated homogenate which were heated for 8 min or more at 66C. The lethality of the come-up period was equivalent to heating the homogenate at 66C for 1.5 min. The homogenate was therefore heated at 66C for the equivalent of 9.5 min (Table 1).

No V. cholerae was recovered from the homogenates heated for 2 min after reaching the temperatures from 71 to 82C. The corrected endpoints at these temperatures ranged from 2.3 to 2.1 min. The minimum heating time used in these studies was 1 min. Since no V. cholerae could be recovered from the homogenates heated for the minimum heating time, the true corrected endpoints were actually less than those calculated. This would also mean that the calculated D values were greater than the true D values.

The D values for each temperature, calculated using the corrected endpoints, ranged from 1.22 min at 66C to less than 0.27 min at 82C (Table 1). The small D values indicated than V. cholerae is very heat sensitive. It has been reported that the organisms is killed after 10 min at 55C (6). This study was probably performed on the classical biotype which is less resistant than the El Tor biotype (4).

Table 1. Endpoints and D values for V. cholerae in Crayfish Meat Homogenate

Temperature (°C)	Endpoint[a] (min.)	Lethality of Come-up Time (min.)	Corrected Endpoint (min.)	D-Value[b] (min.)
66[b]	8	1.5	9.5	1.22
71[b]	1	1.3	2.3	0.30
77[b]	1	1.1	2.1	0.27
82[b]	1	1.1	2.1	0.27

a Endpoint based on no recovery from 15 tubes containing a total of 6.0×10^7 organisms (4×10^6 organisms/tube)

b True D values obtained at these temperatures are less than those recorded due to the minimum test period of 1 minute

Heat penetration. Table 2 shows the lowest temperature recorded in 24 crayfish cooked for up to 10 min in either 100C steam or water. The thermocouples were placed in the crayfish's tail muscle and body cavity, but all of the lowest temperatures were recorded by the thermocouples placed in the animal's body cavity. This indicates that heat penetration occurs at a faster rate in the crayfish's tail muscle than in its body cavity.

Table 2. Effect of Various Cooking Conditions on Internal Temperatures of Crayfish

Thermocouple Placement	Minutes	Lowest Temperature recorded	
		Water (100°C)	Steam (100°C)
Body Cavity	3	62[a]	63[a]
Body Cavity	7	91[b]	89[b]
Body Cavity	10	95[c]	94[c]

a Crayfish weight: 44-48 grams
b Crayfish weight: 38-42 grams
c Crayfish weight: 54-58 grams

Inoculated packs. The cooked crayfish weighed between 18 and 25 g. No V. cholerae were recovered from the cooked crayfish injected with 10^6 V. cholerae in the tail muscle and cooked for 5 min in 100C

water or 100C steam. The organism was not recovered from any of the crayfish which had been injected in the body cavity and cooked in boiling water for 5 min, but the organism was recovered from 2 of the 3 crayfish which had been injected in the body cavity region and cooked in steam for 5 min. Similar results have been reported in research done on crabs injected with the organism and cooked by boiling or steaming (5). Survival of the organism may be due to the slower heat penetration in the crayfish's body cavity or to protection provided by the hepatopancreatic tissue in the body cavity.

A 5 min cook time is employed in most commercial crayfish peeling plants (3). Even though the cephalothorax region in not normally consumed, its liquid and hepatopancreatic tissue often come in contact with the tail meat and may serve as a source of recontamination.

The data in Table 3 indicates that in most cases the slowest heating crayfish cooked in 100C water reached temperatures of 77C and 82C in a shorter period of time than the slowest heating crayfish cooked in 100C steam while steam had the more rapid heating at 60°C and 66°C. These results were also recorded by thermocouples located in the crayfish's body cavity.

Table 3. Time in Minutes Required by the Slowest Heating Crayfish to Reach Internal Temperature

Internal Temperature	Water	Steam
(0°C)	(100°C)	(100°C)
60	3.0	2.5
66	3.5	3.0
71	4.0	4.0
77	4.5	5.0
82	5.0	6.0

The D values for Vibrio cholerae 01 have been established for crayfish meat homogenates. Studies on inoculations of 10^6 V. cholerae in the tail muscle and body cavity of the whole live crayfish and then boiled in 100°C water and 100°C steam for 5 minutes reveal a potential health hazard only in the case of the body cavity inoculations with 100°C steam cooking for 5 minutes.

This potential problem is very rare in Louisiana where the majority of crayfish for human consumption are boiled, i.e. boiling water, add crayfish and timing for 5 minutes upon the return to a boil, whether cooked at home or in commercial peeling plants. Cooking by steam, the recommendation is made that steam under pressure (121°C)

be used for cooking. Thus, the safety of the consuming public will be of concern only if poor manufacturing practices are utilized and which may result in recontamination problems.

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EFFECTS OF VARIOUS CO_2 CONCENTRATIONS ON GROWTH RATE AND FATTY ACID COMPOSITION OF SOME SPOILAGE BACTERIA ISOLATED FROM GULF OF MEXICO BLACK DRUM (*POGONIAS CROMIS*)

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INTRODUCTION

Carbon dioxide has been studied extensively and is currently being used as a means of extending the shelf-life of fresh muscle foods stored under refrigeration. This is of great significance to the fisheries industry since seafoods are among the most perishable commodities and new ways to improve or maintain a high standard of product quality must be found.

In addition the use of CO_2 in modified atmosphere packaging (MAP) presents several more advantages: 1) transportation costs are reduced since ice is replaced by salable product; 2) cross-contamination is avoided and handling is made easier since the product is overwrapped in barrier films or otherwise packaged.

Although CO_2 has been shown to stimulate bacterial growth at low concentrations (Lawlis et al., 1979; Swiencicki and Hartman, 1967; Valley and Rettger, 1927), most recent studies deal with inhibitory effects of CO_2 on bacterial growth and the resulting extension of shelf-life for beef (Clark and Lentz, 1973; Sutherland et al., 1975, 1976), for pork (Adams and Huffman, 1972) and for seafoods (Banks et al., 1980; Brown et al., 1980; Coyne, 1983; Lannelongue et al., 1982; Veranth and Robe 1979).

The degree to which CO_2 affects bacterial growth varies with the types of microorganisms. Some gram-negative as well as gram-positive bacteria can be insensitive to CO_2. However, the most important gram-positive microorganisms are facultative or strict anaerobes, and anaerobic growth is apparently not inhibited by CO_2, whereas the most important gram-negative organisms are strict aerobes and are susceptible to CO_2 inhibition (Gill and Tan, 1980).

The mode of action of CO_2 on bacteria is not fully understood. Several possible mechanisms by which CO_2 inhibits bacterial growth and reproduction have been proposed and/or studied. These include: 1) a decrease in pH due to solubilization and subsequent dissociation of CO_2 at the surface of the system (Banks et al., 1980; King

and Nagel, 1975); 2) a change in the fluidity of the cell membrane leading to dehydration of the bacterial cell (Sears and Eisenberg, 1971); and 3) inhibition of specific enzyme systems (King and Nagel, 1975).

Studies on the effects of CO_2 on bacteria growing in liquid media have also been done. Among the microorganisms studied are *Pseudomonas aeruginosa* (King and Nagel, 1967, 1975), *E. coli* (Repaske and Clayton, 1978), *Pseudomonas fluorescens* (Gill and Tan, 1979), *Alteromonas putrefaciens* and *Yersinia enterocolitica* (Gill and Tan, 1980), *Pseudomonas fragi* and *Bacillus cereus* (Enfors and Molin, 1980).

The purpose of the present study was to measure the growth rate and the changes in fatty acid composition of bacteria grown in atmospheres containing various CO_2 atmospheric concentrations. The microorganisms were isolated from fish that had been stored on ice for up to 9 days.

In this experiment, solid instead of liquid media were used. This presented several advantages: 1) the microorganisms under study were exposed directly to the gaseous atmospheres used; 2) saturation of the media with CO_2 represented no problem; and 3) we simulated in a simplified way what happens when bacteria grow on the surface of a food. "Simplified" because such factors as dominance of a specific microflora or the complex biochemical reactions that occur on animal tissues during spoilage were not taken into account.

MATERIALS AND METHODS

Black drum (*Pogonias cromis*) were harvested at Aransas Pass, Texas. Samples for microbial analyses were taken in triplicate upon landing the fish and after 3, 6 and 9 days of aseptic storage on ice. The microorganisms isolated were identified according to the scheme of Vanderzant and Nickelson (1969).

Two microliters of trypticase soy broth inoculated with each of the bacteria under study were spotted (6 replicates per treatment) on prepoured trypticase soy agar (TSA, Difco) plates. The plates were then placed in low permeability nylon-saran-polyethylene pouches which were evacuated to -1000 mbar and backflushed with each of the atmospheres shown in Table 1.

Table 1. Compositions of gaseous atmospheres used to establish the sensitivity of various bacteria to CO_2.

CODE	CO_2(%)	O_2(%)	N_2(%)
C25	25	20	55
C40	40	20	40
C55	55	20	25
C70	70	20	10
C85)	85	15	--

The atmospheres contained oxygen so as to eliminate anaerobism as a limiting factor for growth. Growth rates of the bacteria were measured as the increase in diameter of the colonies using a micrometer mounted in the eyepiece of a Bausch and Lomb dissection microscope. All colonies were reexposed to air after 6 and 19 days of storage in CO_2 for microorganisms grown at 25 and 4°C, respectively, in order to detect any residual effect CO_2 might have had upon growth. Colony diameters were correlated to optical density and to live number of cells by plating each colony on TSA plates.

The percentage relative inhibition (RI) for each microorganisms at a given CO_2 concentration was calculated as the ratios of the slopes at the maximum growth rate in CO_2 and at the maximum growth rate in air, the growth rate being expressed as the increase in diameter as a function of time.

In order to determine fatty acid compositions, the microorganisms were grown on TSA in large Nalgene sterilizable trays in air, 25% CO_2 and 40% CO_2. The cells were incubated at 25°C and harvested after 18, 48 and 72 hrs, respectively, for each of the atmospheres used. Total lipids were extracted from 2 g cell pellets (wet weight) with methanol, chloroform and water according to the method of Bligh and Dyer (1959). The lipid extract in $CHCl_3$ was then evaporated to dryness under vacuum in a water bath at 40°C. The esterification was performed by refluxing the extract for 30 min. with 5 ml boron trifluoride (12%) in methanol (Supelco). The fatty acid methylesters (FAME) were extracted from the methanol phase with 25 ml hexane, evaporated to dryness and resuspended in 2 ml hexane.

A 1.5 µl aliquot of each sample was injected into a Tracor 560 gas chromotograph equipped with a flame ionization detector and two 6 ft x 4 mm I.D. glass columns. Columns were packed with 3% SE-30 on Chromosorb W-HP (Alltech).

The gas chromatograph operating conditions were the following:

Column temperature: programmed from 150-230°C at 4°C/min, 3 min. initial hold.
Injection and detection ports: 230°C.
Gasflow rates: N2 30 ml/min (60 psig),
H2 34 ml/min (30 psig),
Air 350 ml/min (44 psig).

Individual FAME were identified by comparing the respective retention times to those of a bacterial FAME standard (Supelco). Quantification was done by dividing the area of each acid previously identified by the total area.

RESULTS AND DISCUSSION

In order to measure the growth rate of bacteria on solid media, (determining merely the viability of cells was insufficient), several methods were tested and evaluated. These consisted of: 1)

counting the number of live cells per colony; 2) measuring the optical density of each colony suspended in distilled water; and 3) measuring the diameter of the colonies under a microscope.

The first method resulted impractical because the samples had to be destroyed each time growth measurements had to be taken. Its use was also limited by the fact that although the colonies still expanded, the number of live cells per colony remained constant after 3-4 days at approximately 10^9/colony.

Measuring optical density (OD) was discarded because it also involved destroying the samples and did not seem sensitive enough for slow growing microorganisms (Fig. 1). Expressing growth as the increase in colony diameter correlated well with OD (Fig. 2). It was simple to use, reliable and allowed to follow the growth of the same colonies over the entire duration of the experiment. The same method was used by Kritzman et al. (1977) in their studies to measure growth rates of molds.

Among the microorganisms isolated from black drum at different stages of storage on ice, were *Virbio, Pseudomonas fluorescens, Erwinia herbicola,* and *Moraxella*. The respective growth curves for these microorganisms in CO_2 at 25 and 4°C are shown in Figures 3 through 11.

Among all the microorganisms studied, *Vibrio* was the most sensitive to CO_2 at 25 and 4°C (Figures 3 and 4). At 25°C the relative growth inhibition in 25% CO_2 was 90% while none of the other colonies which had been exposed to higher concentrations of CO_2 showed any signs of growth. Those in 40 and 55% CO_2, however, resumed growth at the same rate as the control, showing no residual effect when exposed to air after 6 days in CO_2 as did colonies that had grown in 25% CO_2. Bacteria stored under 70 and 85% CO_2 did not survive. The effects of CO_2 at 4°C were much more pronounced. None of the colonies grew and only those in 25% CO_2 remained viable. After 19 days, when exposed to air, those colonies showed a long lag phase, showing a clear residual effect by CO_2 on growth (Figure 4).

P. fluorescens showed a much higher resistance to CO_2 at 25°C than did *Vibrio* (Figure 5). Colonies in 25 and 40% CO_2 grew and showed relative inhibiton (RI) rates of 11.2% and 66.3%, respectively. No change in growth rate was observed when these colonies were exposed to air. In concentrations of 55% CO_2 or higher the colonies were completely inhibited but resumed growth immediately upon exposure to air, at a rate approaching that of the control. At 4°C the synergistic effect between low temperatures and CO_2 was evident again (Figure 6). While none of the *P. fluorescens* colonies grew at any CO_2 concentration, all survived and resumed growth after 19 days showing a slight residual effect. *Erwinia herbicola* showed the highest resistance to CO_2. The organism grew well at 25°C in all CO_2 atmospheres, the highest RI rate being 70% in 85% CO_2 (Figure 7). Residual effects were observed for all atmospheres except 85% CO_2 upon exposure of the colonies to air after 7 days

of growth in CO_2. At 4°C, colonies of *E. herbicola* grew in 25 and 40% CO_2 (Figure 8). However, 25% CO_2 seemed to stimulate growth after a lag phase of 4 days. Concentrations of 55% CO_2 and above inhibited growth of colonies of *E. herbicola*. Upon exposure to air after 19 days growth resumed but some residual effect was noticeable which seemed proportional to CO_2 concentration.

Concentrations of 70% CO_2 or more were necessary to prevent growth of *Moraxella* at 25°C (Figure 9). In 85 and 100% CO_2 a high percentage of the bacteria forming the colonies died making growth measuring impossible. A lag phase of 3 days was observed for colonies exposed to 55% CO_2 before growth started. Upon exposure to air, growth of colonies previously in 25 and 40% CO_2 continued at the same rate but no lag phase was seen for colonies previously exposed to 55 and 70% CO_2. In contrast to *P. fluorescens* which proved to be more sensitive than *Moraxella* upon exposure to CO_2 at 25°C, the opposite effect occurred at 4°C (Figure 10). No growth was observed in any of the atmospheres containing CO_2. Colonies in 25% CO_2 resumed growth after 19 days when exposed to air while those in 40% CO_2 survived only partially.

In order to determine the effects of CO_2 in the presence/absence of CO_2, colonies of *P. fluorescens* (Figure 11) and *Moraxella* (Figure 12) were grown in atmospheres containing 6.3 and 12.1% CO_2 in N_2, referred to as CN1 and CN2, respectively. Growth rates were compared with colonies grown in air and in 25% CO_2 (C25). Lack of 02 seemed to be the critical factor in the growth inhibition of both microorganisms, particularly *Moraxella* where an increase in the concentration of CO_2 from 6.3 to 12.1% had practically no effect (Figure 12). For *P. fluorescens* an increase in atmospheric CO_2 resulted in an additional inhibitory effect (Figure 11). No residual effect whatsoever was apparent.

The plots of relative inhibition for *Moraxella* and *P. fluorescens* as a function of CO_2 concentration at 25°C are shown in Figure 13). The RI for *P. fluorescens* was only 11% at 25% CO_2, but increased rapidly to 66% in 40% CO_2 and to 100% at 55% CO_2. In contrast, Gill and Tan (1979) observed a RI of 38% for *P. fluorescens* growing in 9.9% CO_2 and a maximum RI of 48% in 33% CO_2. This indicates that growth inhibition by CO_2 was higher on solid than in liquid media probably due to direct exposure of the microorganisms to CO_2.

The increase of the rate of inhibition was more gradual for *Moraxella* than it was for *P. fluorescens*. Complete growth inhibition of *Moraxella* required 70% CO_2.

As reported by Enfors and Molin (1981), there is a synergistic effect on growth inhibition of bacteria between low temperature and CO_2. Comparable observations resulted from our experiments with *Moraxella* and *P. fluorescens* (Figure 14) when the growth rate of these microorganisms was measured in 25% CO_2 at temperatures of 4, 10, 15, 20 and 25°C. In contrast, however, to what Enfors and Molin (1981) observed with *P. fragi*, the increase in relative

growth inhibition as temperatures decreased was not linear and no correlation between increased RI and CO_2 solubility at those temperatures was found.

Table 2 shows the effects of 25 and 40% CO_2 on fatty acid composition of lipid fractions from *Vibrio*, *P. fluorescens* and *Moraxella*. An overall increase in unsaturation from hexadecanoic (C16:0) to hexadecanoic (C16:1) from octadecanoic (C18:0) to octadecenoic (C18:1) was observed in all cases. In addition a reduction in the number of carbons of C18 to C16 fatty acid resulted in *Vibrio* and *Moraxella*. The change in chain length did not seem significant in *P. fluorescens*. This would indicate that an alteration of the permeability of the cell membrane occurs that is probably not immediately reversible. This may account for the fact that *Vibrio* grown in 25% CO_2 and *Moraxella* and *P. fluorescens* grown in 25% CO_2 and 40% CO_2 did not resume growth as rapidly when exposed to air as those colonies that had been completely inhibited (Figures 3, 5, 9). Some other reversible effect must be responsible for complete growth inhibition in higher CO_2 concentrations where growth resumes at its maximum rate when colonies are reexposed to air.

CONCLUSIONS

This study gives a good representation of the effects of CO_2 on bacterial growth. Among the conclusions that can be drawn are: 1) refrigeration is essential if CO_2 is to be effective in MAP on fresh muscle foods; 2) lower CO_2 concentrations are needed as temperatures decrease to achieve complete bacterial inhibition; 3) spoilage will probably resume at or near the same rate once the food is reexposed to air; and 4) inhibition of bacterial growth is caused by several factors one of which is alteration of enzymatic reactions leading to changes in the fatty acid composition of bacterial cell membranes.

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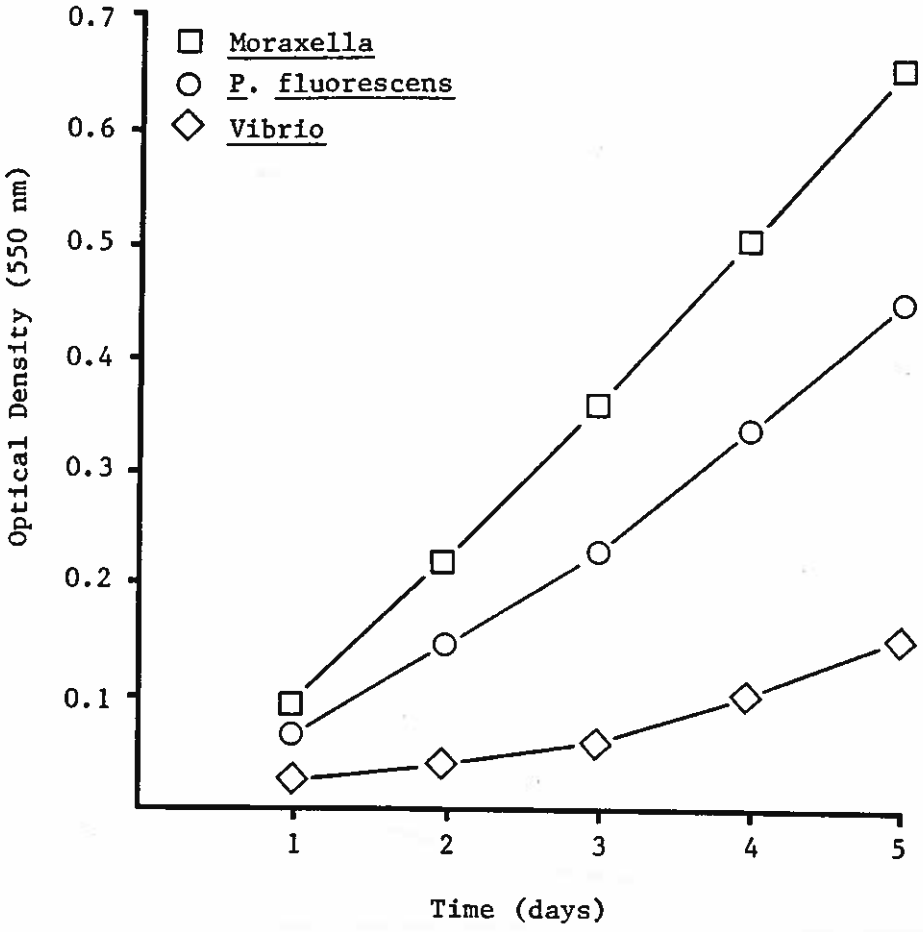

Fig.1 - Optical density measurements (OD 550) of colonies of Moraxella, P. fluorescens and Vibrio suspended in 4 ml distilled water.

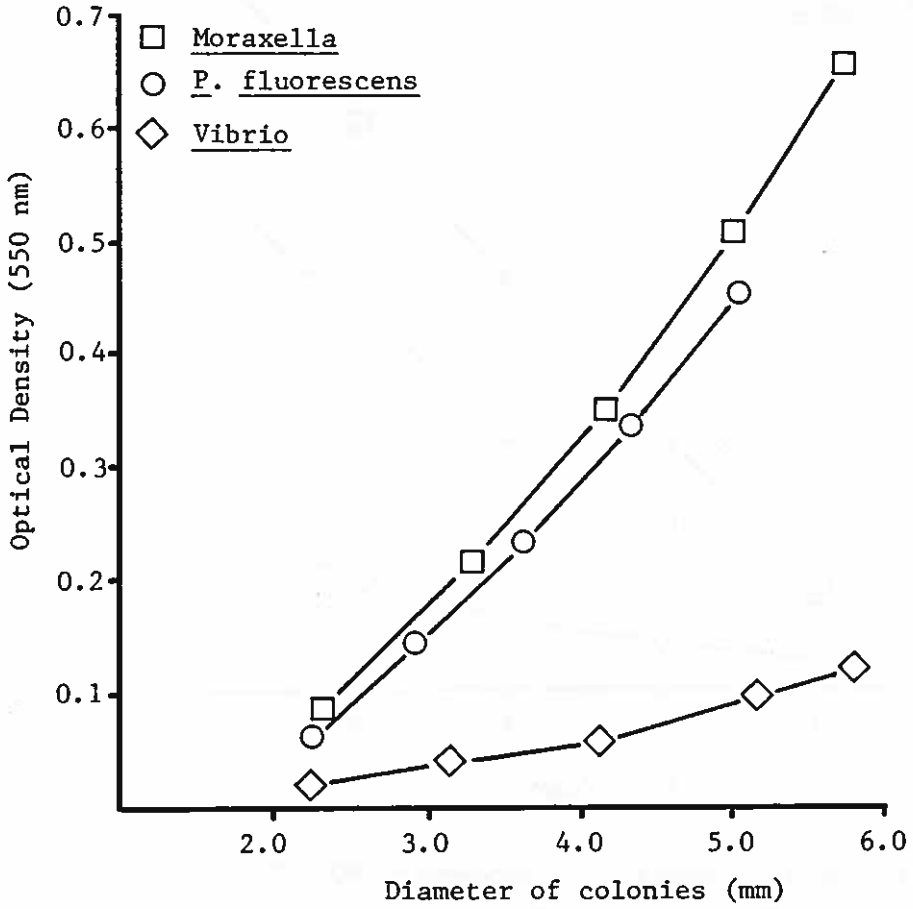

Fig.2 - Diameter vs optical density (OD 550) of colonies of Moraxella, P. fluorescens and Vibrio suspended in 4 ml distilled water.

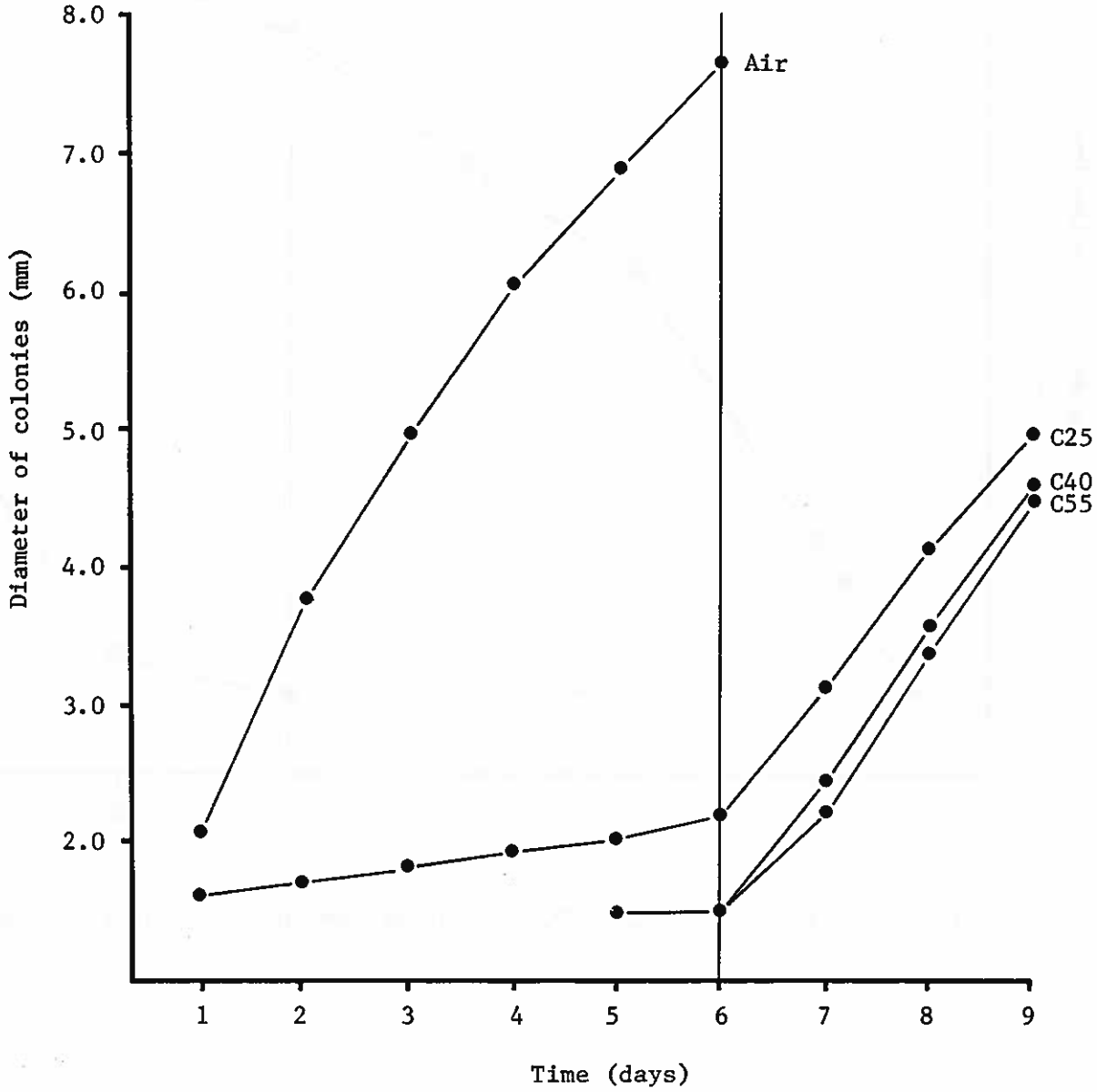

Fig.3 - Effects of various CO_2 concentrations on the growth of Vibrio at 25°C.

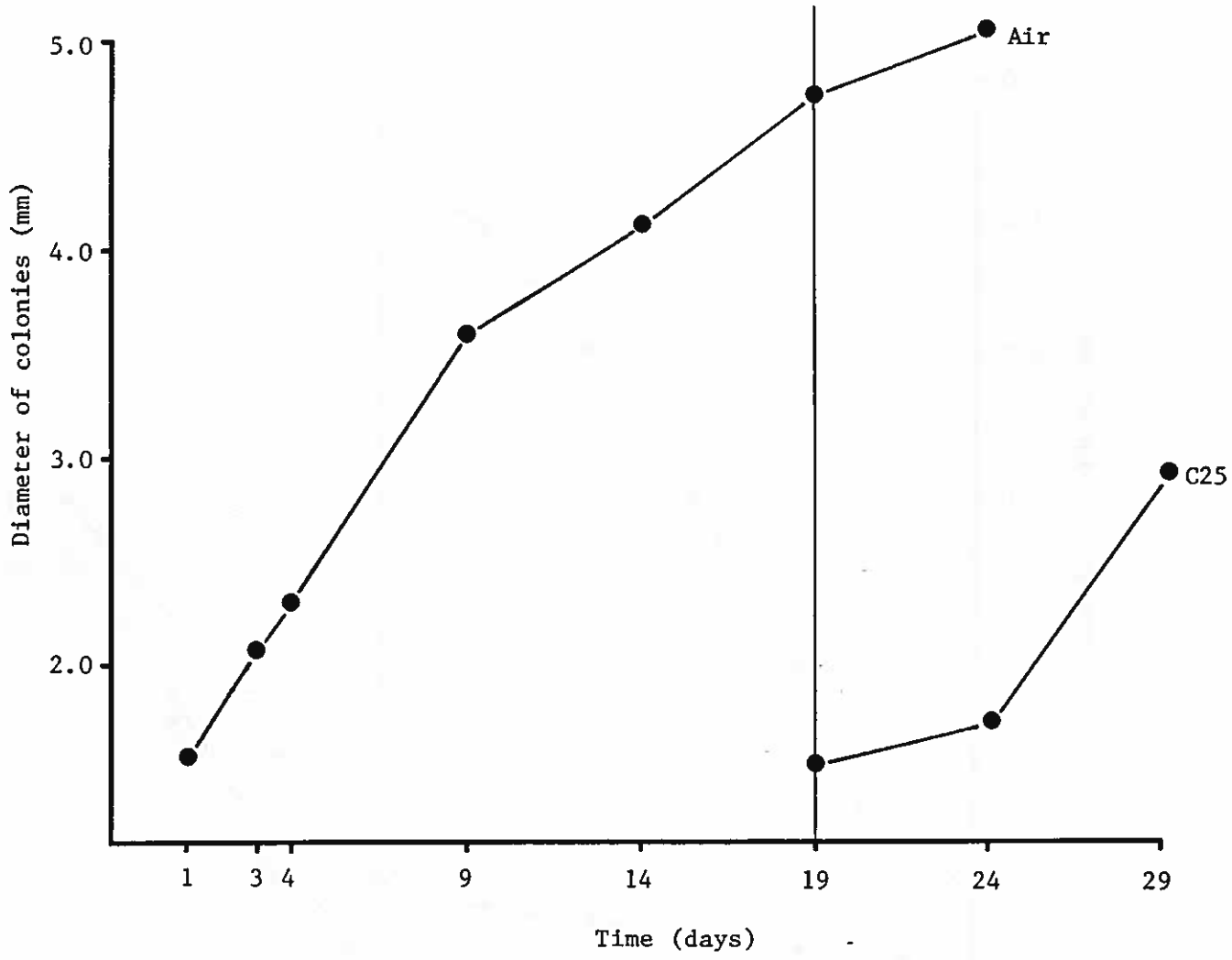

Fig.4 - Effects of various CO_2 concentrations on growth of Vibrio at 4°C.

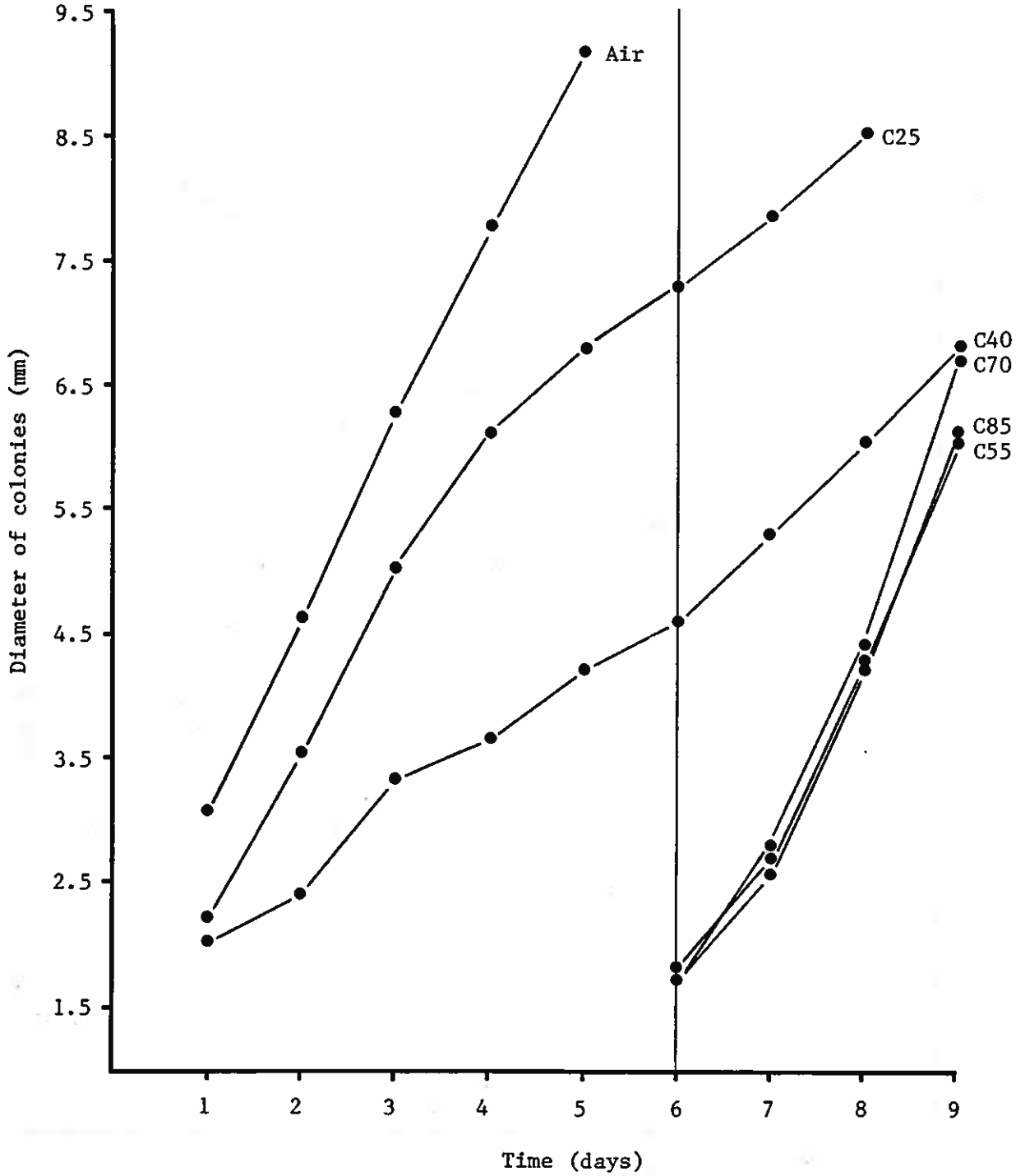

Fig.5 - Effects of various CO_2 concentrations on growth of Pseudomonas fluorescens at 25°C.

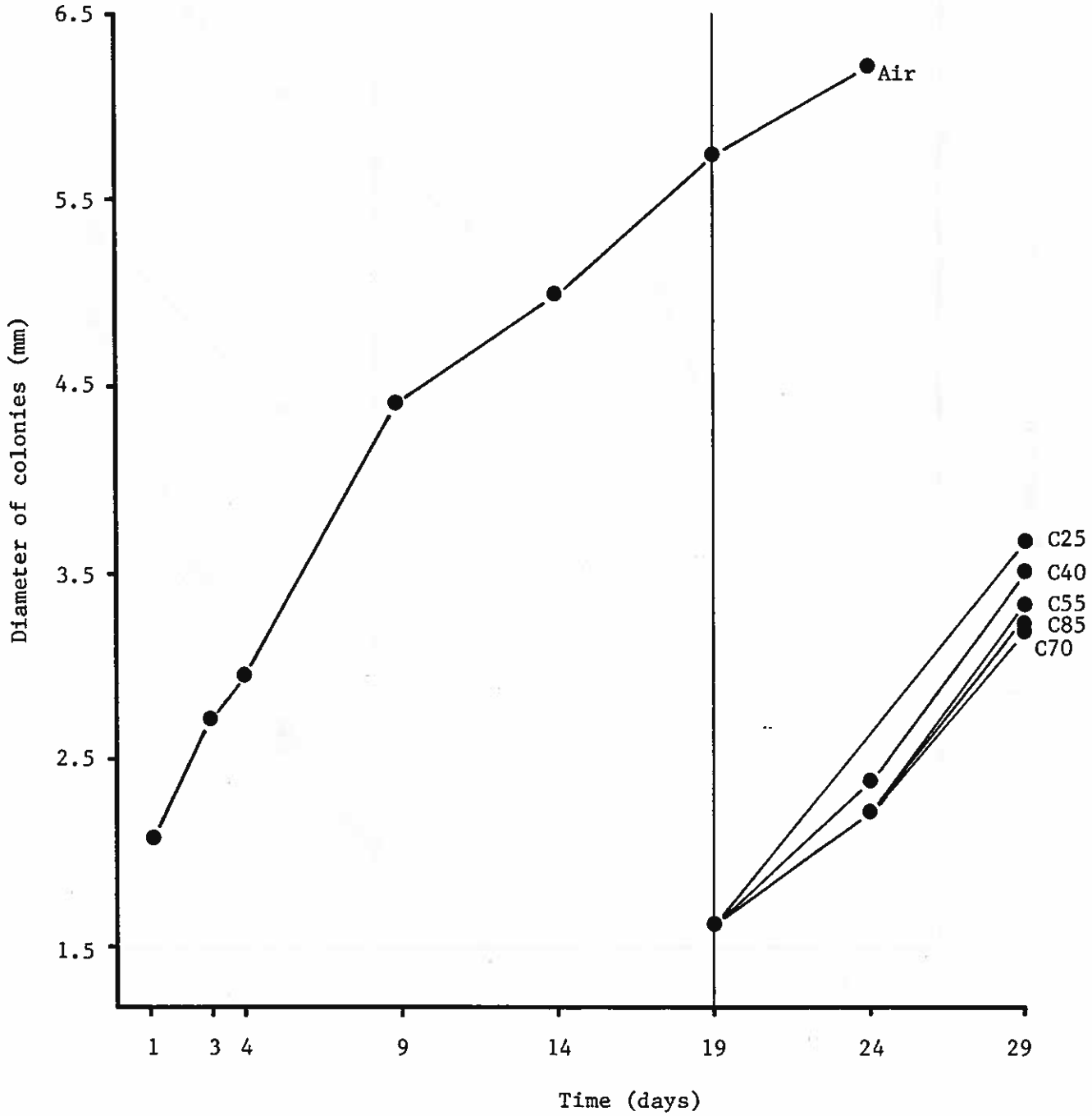

Fig.6 - Effects of various CO_2 concentrations on growth rate of Pseudomonas fluorescens at 4°C.

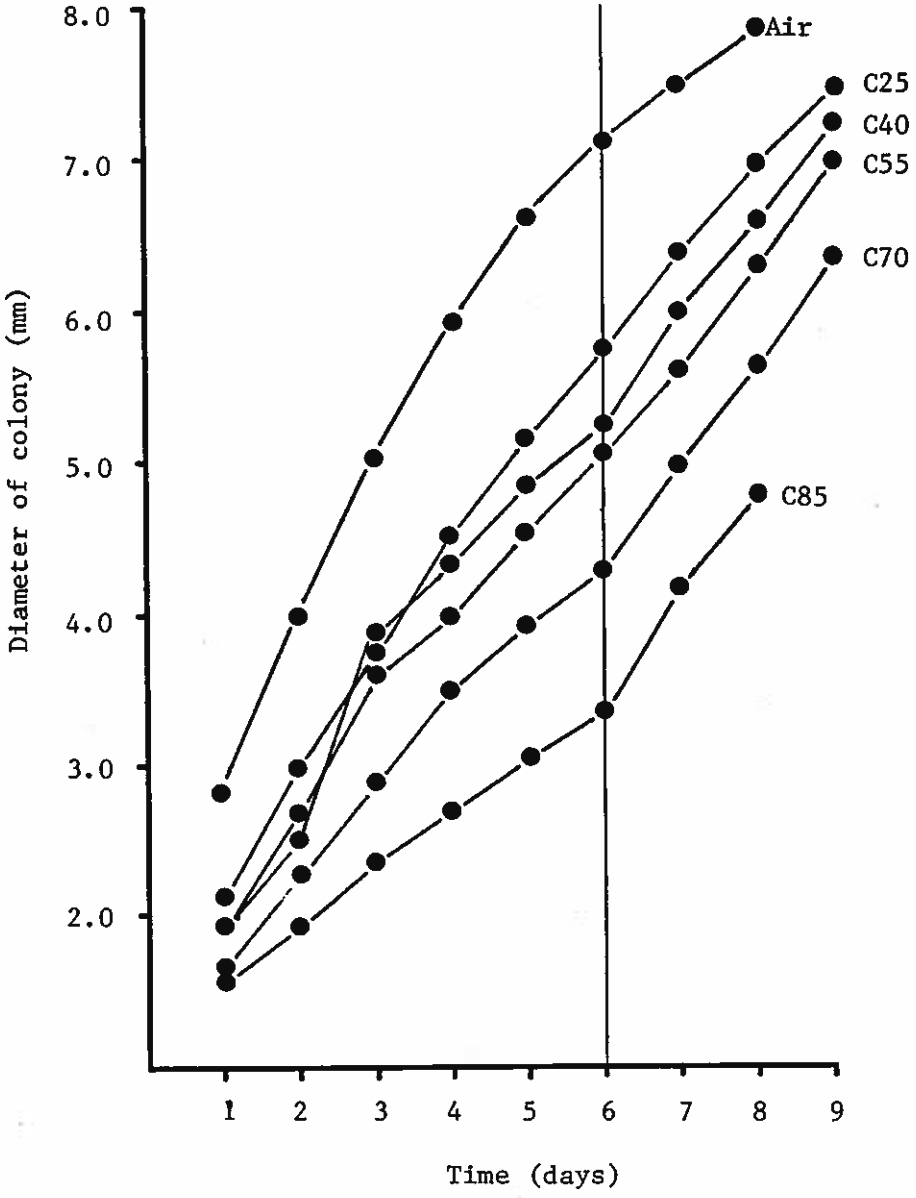

Fig.7 - Effects of various CO_2 concentrations on growth of Erwinia herbicola at 25 °C.

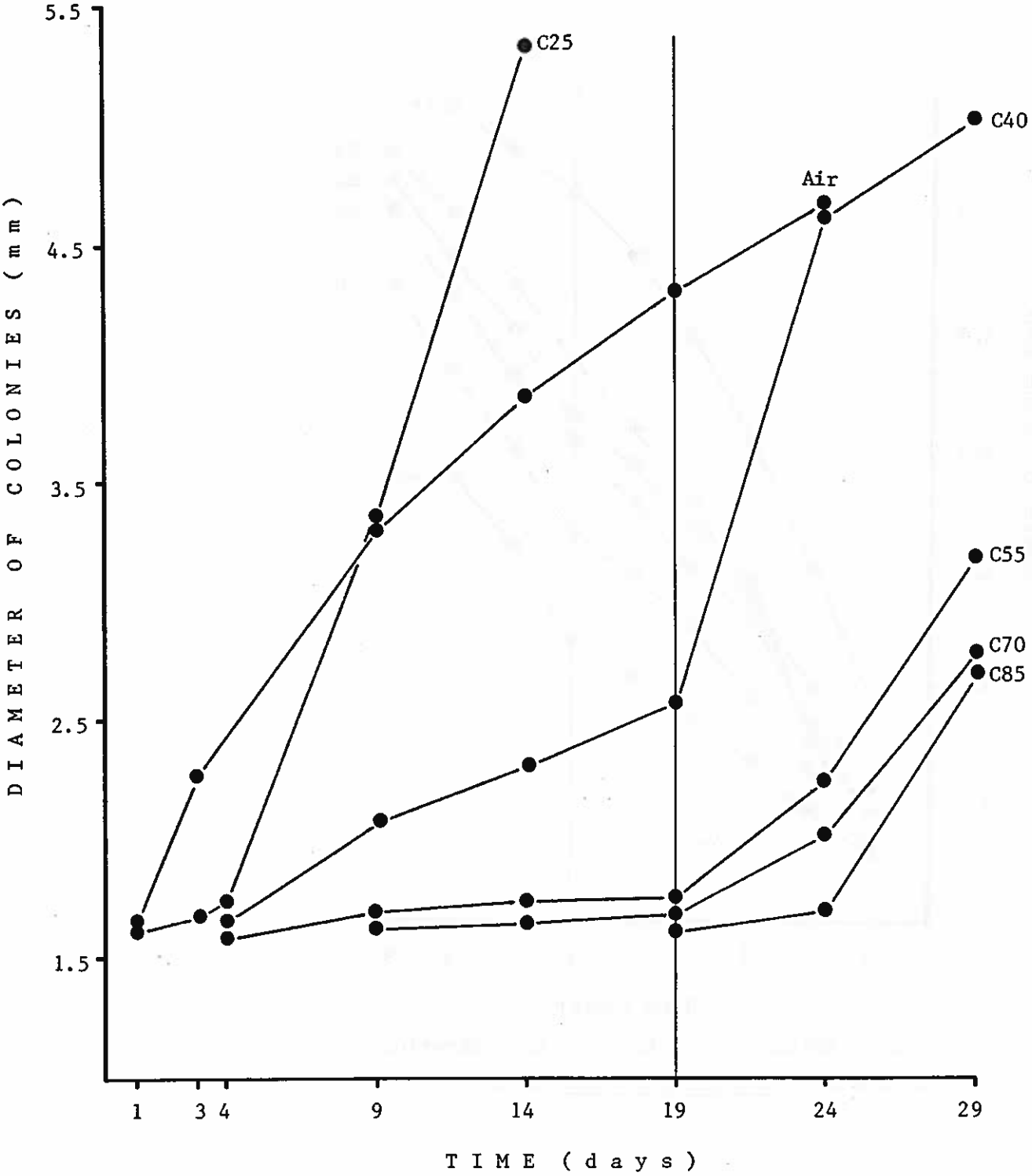

FIG.8 - EFFECTS OF VARIOUS CO_2 CONCENTRATIONS ON GROWTH RATE OF ERWINIA HERBICOLA AT 4°C.

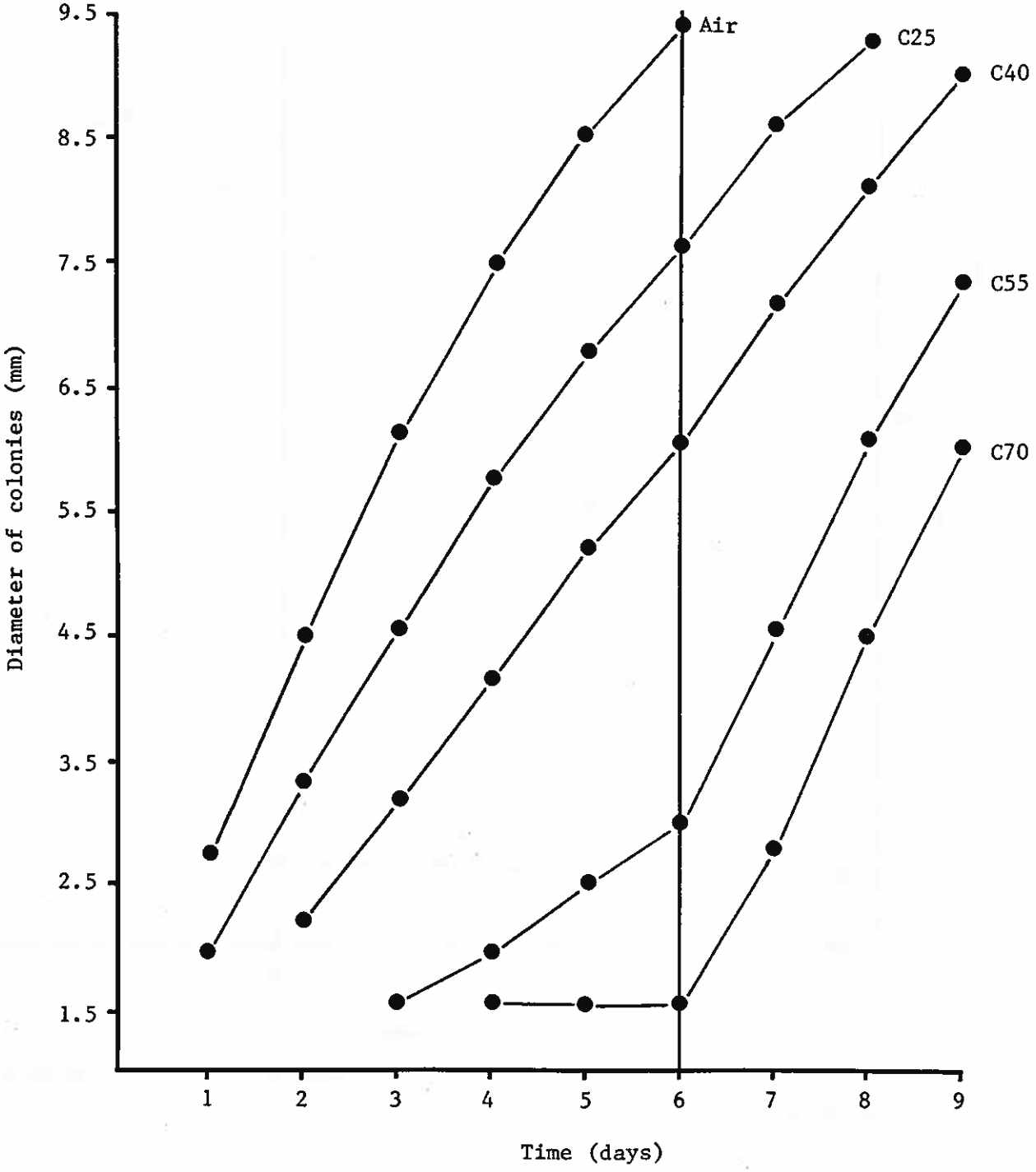

Fig.9 - Effects of various CO_2 concentrations on growth of Moraxella at 25°C.

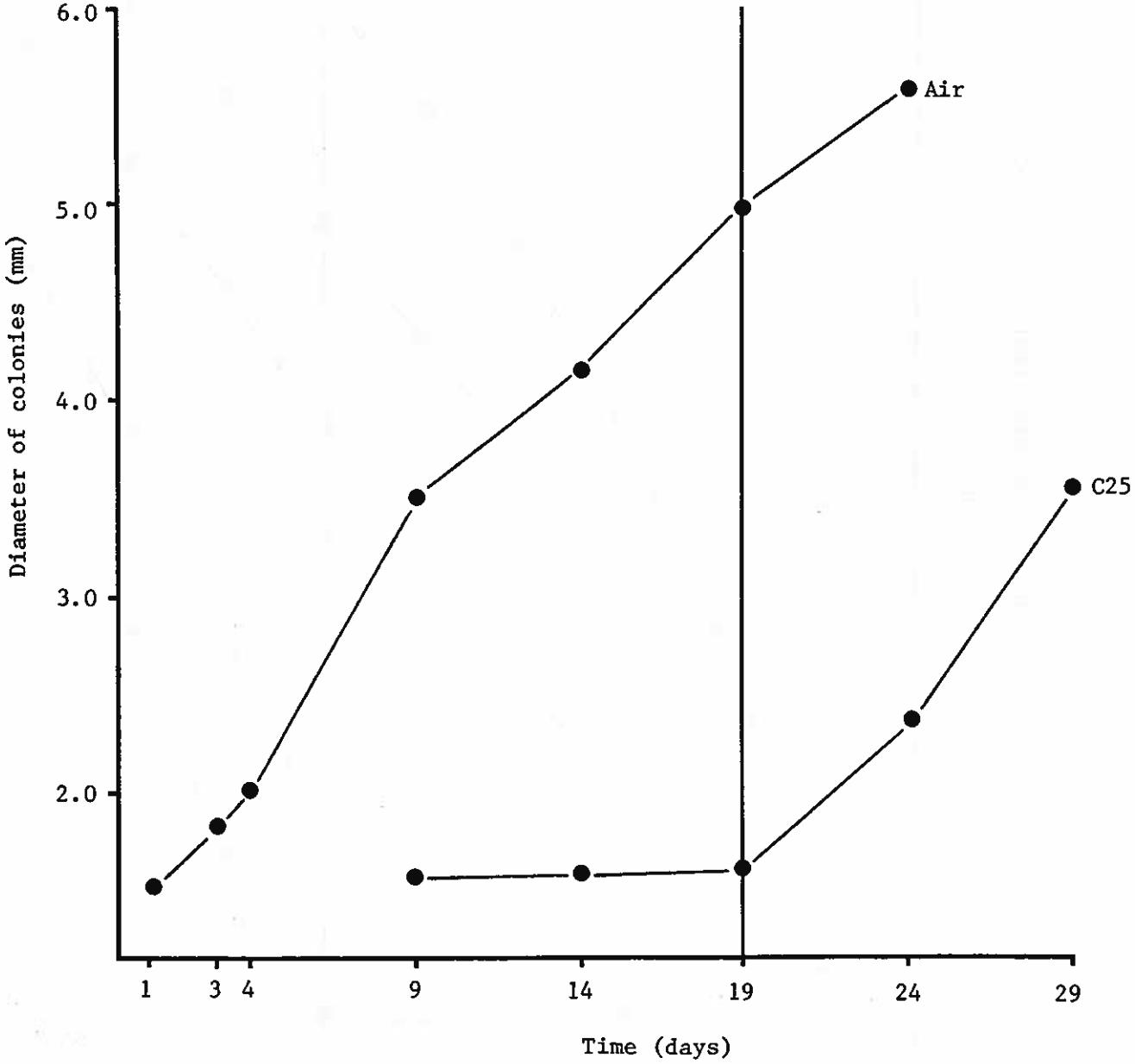

Fig.10 - Effects of various CO_2 concentrations on growth of Moraxella at 4°C.

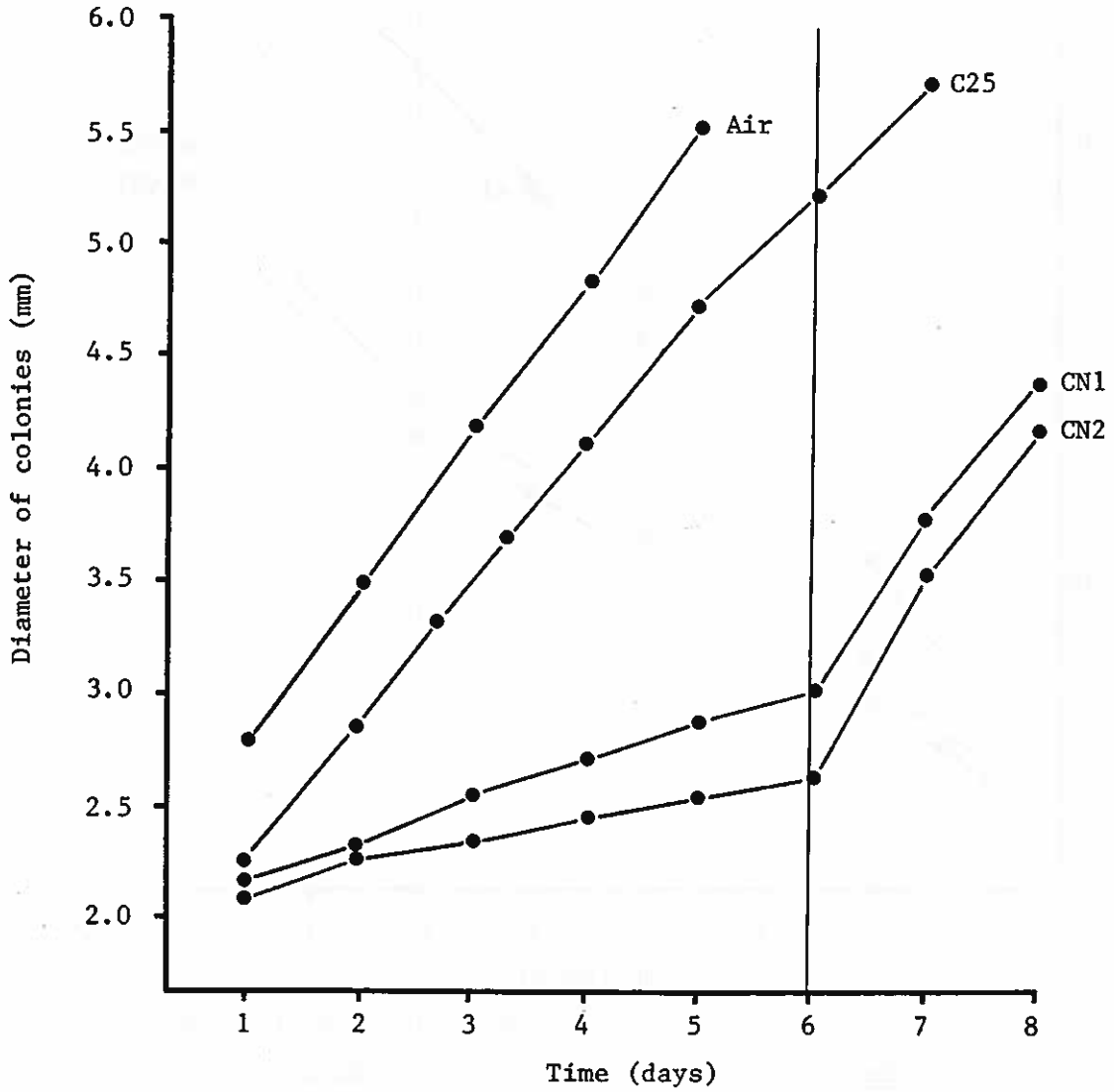

Fig.11 - Effects of CO_2 in the presence/absence of O_2 on growth rate of P. fluorescens. (C25: 25% CO_2-20% O_2-55% N_2; CN1: 6.3%CO_2 in N_2; CN2: 12.1% CO_2 in N_2)

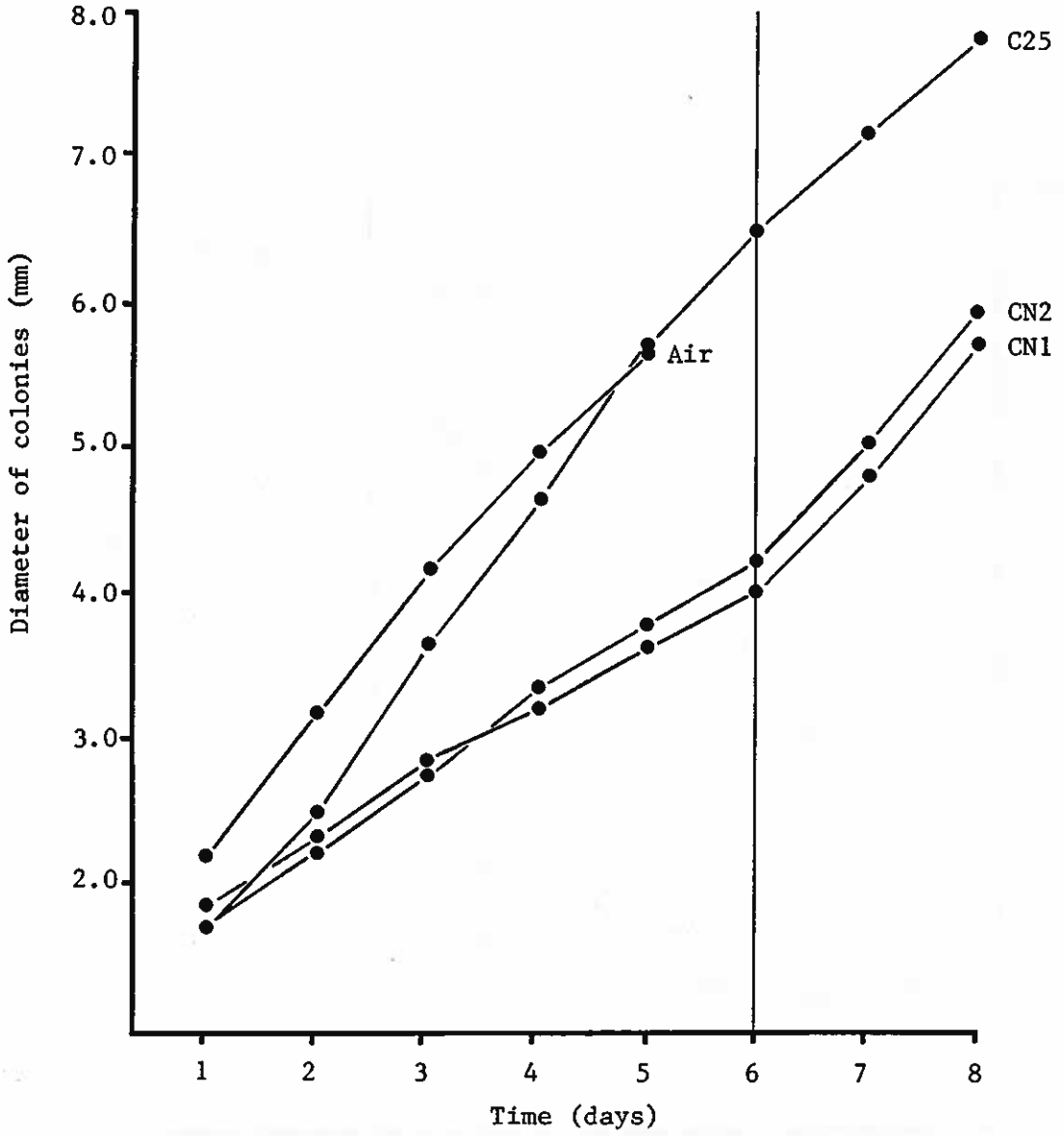

Fig.12 - Effects of CO_2 in the presence/absence of O_2 on growth of Moraxella . (C25: 25% CO_2-20% O_2-55% N_2; CN1: 6.3% CO_2 in N_2; CN2: 12.1% CO_2 in N_2)

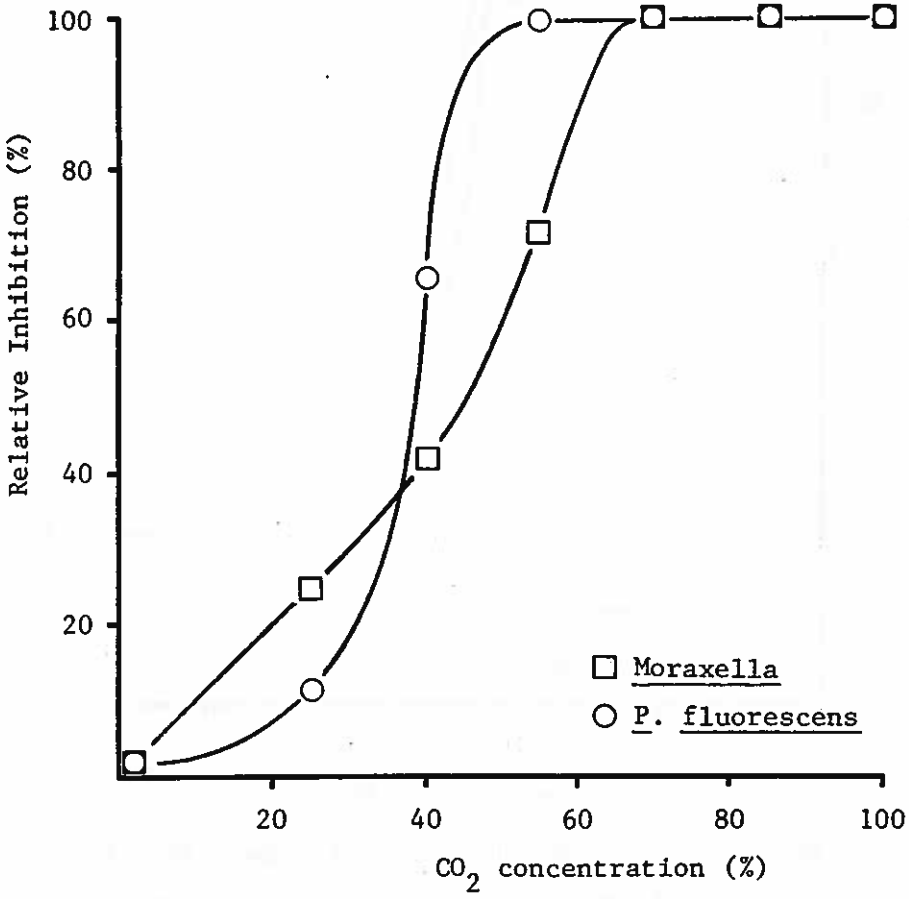

Fig.13 - Relative inhibition of growth as a function of CO_2 concentration at 25°C.

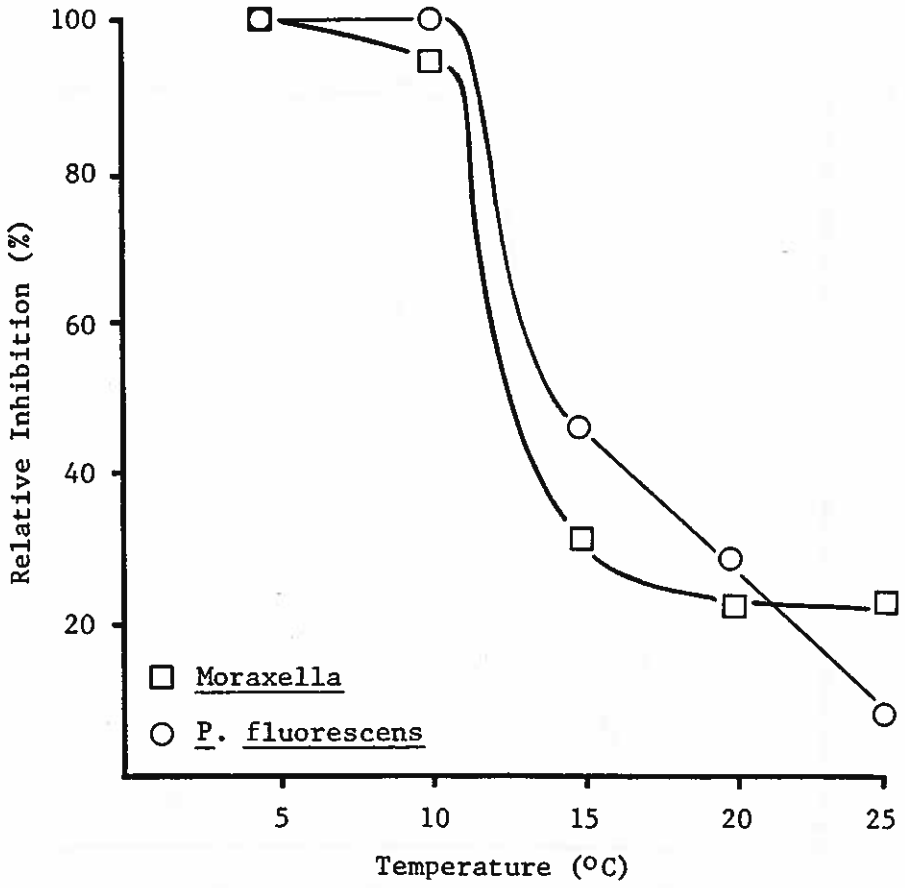

Fig.14 - Relative inhibition of growth as a function of temperature in an atmosphere containing 25% CO_2-20% O_2-55% N_2.

Table 2. Fatty acid composition of Moraxella, P. fluorescens and Vibrio grown at 25°C in air and in atmospheres containing 25% CO_2 (C25) and 40% CO_2 (C40).

Fatty acid	Moraxella			P. fluorescens			Vibrio		
	Air	C25	C40	Air	C25	C40	Air	C25	C40
12:0				-	-	-	0.5	-	-
14:0	2.1	1.2	3.8	1.5	0.4	0.4	3.4	1.0	0.7
15:0	1.4	1.7	4.6	1.8	0.4	0.5	3.8	1.0	0.9
16:0	14.8	10.6	20.0	24.3	20.9	20.5	18.2	19.1	19.0
16:1	9.0	30.7	11.8	27.0	28.7	21.0	18.4	32.9	28.9
17:0	3.8	1.6	11.5	1.3	-	-	7.0	1.2	1.3
a-17:0	5.5	7.6	-	-	-	-	-	1.5	1.7
17:0 cyc	-	-	-	2.9	11.9	13.7	-	-	-
18:0	17.4	7.0	16.8	9.0	-	-	15.7	6.3	9.4
18:1	22.1	37.9	15.6	22.7	32.6	31.5	22.5	31.3	29.4
18:2	-	-	-	1.5	0.7	0.9	-	-	1.8
20:1	-	-	-	-	1.0	1.2	-	2.2	2.3

Key to fatty acid designation: the figure before the colon indicates the number of carbon atoms in the fatty acid chain; the one after the colon denotes the number of double bonds.

a-17:0: 14 - methylhexadecanoate

17:0 cyc: cis - 9, 10 - methylene hexadecanoate

Table 3. Ratios of the degrees of unsaturation and chain length of fatty acids from Moraxella, P. fluorescens and Vibrio.

	Moraxella			P. fluorescens			Vibrio		
	Air	C25	C40	Air	C25	C40	Air	C25	C40
Degree of unsaturation: $\frac{C16:1 + C18:1}{C16:0 + C18:0}$	0.97	3.90	0.74	1.49	2.93	2.56	1.21	2.53	2.05
Chain length: $\frac{C18:0 + C18:1}{C16:0 + C16:1}$	1.66	1.09	1.02	0.62	0.66	0.76	1.04	0.72	0.81

SURVIVAL OF SALMONELLA IN EXPERIMENTALLY CONTAMINATED BREADED SHRIMP DURING DEEP FAT FRYING

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INTRODUCTION

Despite increased federal regulatory effort in removing foods from commerce through seizures and destruction since 1967, the presence of Salmonella in foods remains a significant public health problem. Increased federal and state compliance efforts have put a considerable burden on industry while showing little or no effect in reducing the incidence of salmonellosis (1,2).

Raw meat and poultry, known sources of salmonellae, and poorly cooked products account for the majority of reported outbreaks of salmonellosis (2,3,4). A review of several documented outbreaks traced to pre-cooked beef roasts showed that the common internal cooking temperature of less that 130 F failed to destroy salmonellae (4). Salmonella survived an internal temperature of 141.5 F in beef roasts experimentally injected with $2X10^7$ cells per roast (5). Chicken portions inoculated with $1.3X10^6$ cells per g and sealed in boilable pouches were salmonellae free after 10 min of boiling at an internal temperature of 179 F. However, Salmonella was recovered after 8 min at a terminal temperature of 169 F (6). In another plastic pouch cooking study on turkey (7), heat resistant S. senftenberg 775W was detected at final processing temperatures of 159 and 163 F but not at 164 and 164.8F. That study recommended a final temperature of at least 165 F in the zone of slowest heating. A terminal temperature of 160 F was proposed as adequate to destroy salmonellae in a test of 24 baked goods (8). Ritter et al. (9) reported that temperatures achieved in slow cookers were sufficient to eliminate Salmonella even at the minimal recommended cooking time. In that study the inoculum size was $2X10^8$ cells per recipe, and the minimum temperature achieved in any of the meals was 155 F.

While red meat and poultry harbor salmonellae, there is little evidence of the natural occurrence of salmonellae in shrimp. Handling, processing, and transportation are sites at which cross contamination of the product may occur. Indeed, seizures by the Food and Drug Administration of Asiatic shrimp imported into the United States indicate poor sanitation in the handling of shrimp in those countries (10).

This analysis was undertaken as a consumer risk assessment of DOC inspected shrimp to determine the internal cooking time-temperature conditions necessary to destroy Salmonella in frozen, raw-breaded shrimp and to ascertain if these conditions could be achieved by cooking to recommended consumer label instructions.

MATERIALS AND METHODS

Shrimp sizes

The two sizes of shrimp used were 12 to 15 count per pound (12/15) raw breaded round and 31/35 breaded mini-rounds. All shrimp were hand breaded by and purchased from a local processor.

Salmonella serotypes

Five serotypes were used in the thermal study: *S. senftenberg 775W*, *S. senftenberg*, *S. weltevreden*, *S. typhimurium*, and *S. lexington*. FDA supplied the latter four serotypes isolated from imported shrimp, and the *775W* was supplied by Dr. Peter K. Stocks, University of Southern Mississippi.

Cell concentrations

Two ml of culture grown in lactose broth for 24 hr was inoculated into 200 ml tryptic soy broth and incubated 24 hr @ 35 C. The culture was then centrifuged in a Beckman J2-21 centrifuge @ 4 C. After initial concentration the cells were washed and centrifuged three times in Butterfield's phosphate buffer and diluted to an absorbence reading of 0.46 to 0.56 on a Bausch and Lomb Spectronic 20 set at 550 nm. Total aerobic plate counts (TAPC) were run on that dilution to determine the number of viable *Salmonella* at the absorbence reading.

Injection levels and site

Average weights of the two shrimp sizes were 30.5 g for the 12/15 count and 8.3 g for the 31/35 count. Appropriate dilutions were made of the concentrated cell culture to inject each shrimp at its center with approximately $1X10^6$ cells per g in a volume near 0.05 ml. Shrimp were thawed to 20 C to facilitate injection and then refrozen overnight.

Cooking and thermal monitoring

A review of DOC inspected product label cooking instructions indicated that the most common recommendation for raw breaded shrimp was 3-4 min at 350 F. Pretrial cooks indicated that frying 3 min at 350 F for the 12/15 count and 2 min at 350 F for the 31/35 count were adequate for proper cooked appearance.

Internal shrimp temperatures were recorded during all cooks with a Leeds and Northrup Speedomax 165 recorder using O.F. Ecklund CNS 2-1/4" thermocouples. A 1/16" diameter hole was drilled to the center of four shrimp per cook for connection to the thermocouples.

Sample unit pull times

After overnight freezing of injected shrimp, initial plate counts (To) were performed prior to each run. Shrimp were drawn from the fryer baskets at 2, 3, 3.5, 4, 5, and 6 min for the 12/15 count shrimp. The cook was discontinued at 3 min, and the basket of shrimp was allowed to drain over the hot oil. The 3.5 min sample signifies 0.5 min of

drain time, the 4 min is 1 min of drain time, etc. The cook for 31/35 count was discontinued at 2 min with additional sample pulls during drain time.

Microbiological procedures

Both quantitative and qualitative analyses were performed on all cooks with all serotypes. Three shrimp were drawn per pull time for the quantitative analysis. The shrimp used in the quantitative analysis were blended in phosphate buffer at a 1:10 dilution and plated directly on BGS agar as described by Sims, Kelley, and Foltz (11). The only modification to the procedure was the plating of 0.25 ml of diluent instead of 0.1 ml. The qualitative analysis was performed in accordance with FDA BAM (12).

Statistical Analysis

Sequential polynomial regression model building least squares procedures were used to estimate kill time, temperature at kill time and the standard error of this estimate for each serotype/shrimp size combination.

RESULTS AND DISCUSSION

Both quantitative and qualitative data were obtained for all serotypes, shrimp sizes, and pull times. Quantitative results were used to statistically estimate cooking times and temperatures necessary to destroy all injected Salmonella. The more sensitive qualitative results indicated actual times at which Salmonella was no longer recovered.

Statistical analysis of the quantitative data showed that 1) the surviving number of Salmonella was highly related to cooking time, 2) temperature was strongly related to time, 3) duplicate runs in the same serotype and shrimp size were not significantly different. A polynomial model of maximum degree two relating number of salmonellae and time was determined by conventional least squares regression techniques. Such an equation was computed for each serotype and each shrimp size using the quantitative data from duplicate runs. By letting the number salmonellae (y) equal zero in this equation, the time (x) necessary to kill all salmonellae was estimated. With the death time calculated, a different polynomial model specific to each serotype and shrimp size relating time to temperature was determined to estimate the temperature at this time. Table 1 shows the estimated times, temperatures, and temperature standard errors for the 12/15 and 31/35 shrimp. Results of the qualitative method to detect presence or absence of Salmonella are presented in Table 2.

All Salmonella serotypes were destroyed in less than 4.5 min in the 12/15 shrimp when temperatures reached near 180 F. In the 31/35 size the time to kill was less than 2 min with temperatures up to 225 F. The higher estimated death temperature in smaller shrimp could be expected

because of the increased rate at which these shrimp cooked. There was more variation among serotype kill times and temperatures in the 31/35 count than in the 12/15 count. Salmonella injection and temperature probe insertion to an approximate geometric center is less critical in large shrimp. Small deviations from this location produce greater differences in kill times and temperatures for smaller shrimp. There was no correlation between serotypes and heat sensitivity when comparing the two sizes of shrimp cooked. Even S. senftenberg 775W was no more heat resistant in this study than the other serotypes.

Table 1 indicates there should be no Salmonella survival for 31/35 shrimp at 2 min, the time at which cooking was terminated; however, the qualitative method run simultaneously detected Salmonella in 3 of the 10 runs (Table 2). This method showed no survival at 2.5 min, 30 sec of drain time.

The importance of the drain time in Salmonella lethality is even more dramatic in the 12/15 shrimp where Salmonella survived the 3 min cook in all 10 runs. There was recovery even at 4 min (1 min of drain time) in 8 of the 10 runs. However, no detection was made at a total of 5 min. Because of the insulative quality of breading ingredients, shrimp temperatures continued to rise a full 3 min after removal from the fryer with an average internal increase of 34.5 F. Thus, in both shrimp sizes, the post-cooking temperature rise was critical in the destruction of Salmonella.

The determination of an exact thermal death time for Salmonella sp. was not the intent of this risk assessment analysis. Rather, what has been found is a small range of both times and temperatures over which the five serotypes selected have been killed under the "worst case" contamination employed in this study. The range is understandable considering the variables within the experiment, e.g., location of the geometric centers of all shrimp, To differences among serotypes, and the lack of exactness in the quantitative microbiology.

The other issue addressed in this experiment was the efficacy of consumer frying methods in eliminating Salmonella. Again, under the "worst case" analysis conducted, Salmonella was not always destroyed during frying, especially in the large shrimp. However, under normal length drain times, the post-cooking heat increase did eliminate all Salmonella, thus offering reasonable consumer protection.

Table 1. Estimates of cooking times and temperatures lethal to Salmonella.

31/35

Serotype	To	Time to kill (min)	Temp at kill (^{o}F)	Temp Standard Error
S. typhimuirum	$4.7x10^5$	1.75	204	8.2
S. lexington	$4.1x10^5$	1.44	212	10.8
S. weltevreden	$3.2x10^5$	1.77	171	12.8
S. senftenberg	$8.8x10^5$	1.91	225	10.4
S. senftenberg 775W	$8.4x10^5$	1.82	200	5.8

12/15

S. typhimurium	$1.1x10^6$	4.32	180	8.9
S. lexington	$4.2x10^5$	4.36	180	3.1
S. weltevreden	$3.6x10^5$	4.39	174	7.0
S. senftenberg	$2.0x10^5$	4.17	180	7.4
*S. senftenberg 775W	$8.7x10^4$	4.05	174	4.0
*S. senftenberg 775W	$1.4x10^6$	4.34	178	7.2

*Run on consecutive days with different To: data not combined as in duplicate runs of other serotypes with the same To.

Table 2. Recovery of Salmonella by enrichment method.

31/35

Serotype	Run	Time (min)				Drain	
		0.5	1.0	1.5	2.0	2.5	3.0
S. typhimurium	(1)	+	+	+			
"	(2)	+	+				
S. lexington		+	+	+			
"		+	+	+			
S. weltevreden		+	+	+			
"		+	+				
S. senftenberg		+	+	+	+		
"		+	+	+	+		
S. senftenberg 775W		+	+	+	+		
"		+	+	+			

12/15

		2.0	2.5	3.0	3.5	4	5	6
S. typhimurium		+	+	+	+	+		
"		+	+	+	+	+		
S. lexington		+	+	+	+	+		
"		+	+	+	+	+		
S. weltevreden		+	+	+	+			
"		+	+	+	+	+		
S. senftenberg		+	+	+	+	+		
"		+	+	+	+	+		
S. senftenberg 775W		+	+	+	+			
"		+	+	+	+	+		

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IN PLANT USE OF A MINI-COMPUTER AND A PORTABLE PYROMETER TO MONITOR HEAT PENETRATION OF RETORTED LIVE BLUE CRABS AND PASTEURIZED CANNED CRAB MEAT

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INTRODUCTION

Thermal processing is an important aspect of the blue crab industry. Most Atlantic coast blue crab processors rely on pressurized steam to retort live blue crabs prior to removing cooked meat from the crab bodies. The recovered meat, which is usually handpicked, is then marketed as "fresh" crab meat with a refrigerated shelflife of 10 - 14 days or is canned and pasteurized in a hot water bath to produce a refrigerated product with a shelflife of greater than six months. Both the retorting and pasteurization processes require controlled time/temperature relationships to insure product safety, quality, and yield.

Several hundred to several thousands pounds of live blue crabs are cooked in large horizontal or vertical steam pressure retorts at 15 pounds/in^2 to an internal temperature of 235°F (112.8°C). If the crabs are cooked at a lower temperature, the meat is too moist, difficult to pick, has excessive bacterial populations, and a short shelflife. Too long a cook excessively dries the meat and reduces the percent yield. The cooked crabs are backed and the meat is removed by trained pickers or less frequently by a crab picking machine. The meat can then be packed as "fresh" or be placed in cans for hot water pasteurization. Crab meat was traditionally hot water (186 - 190°F, 85.6 - 87.8°C) pasteurized in one pound number 401 cans to an internal temperature of 185°F (85°C). Traditional blue crab meat pasteurization specifications require that the 185°F (85°C) temperature be maintained at the geometric center of the can for one minute (3). Processing for too short a period or at lower temperatures causes the meat to be undercooked which usually results in early spoilage of the product and the potential development of toxins produced by type E Clostridium botulinum. Cooking at an excessive temperature or for an extended period causes the meat to turn blue. The blue coloration is not harmful, but the meat is usually unacceptable to the consumer (1, 6, and 7).

Over the last several years, blue crab processors have come under increasing pressure from consumers, state and federal regulatory agencies,

and from within the industry itself to improve product quality and safety. The National Blue Crab Industry Association adopted industry wide pasteurization guidelines in June 1983 that detailed specific equipment and processing requirements for pasteurized meat. Rising energy and raw material costs have increased the financial incentive of processors to establish energy conservation measures and improve product yields.

Several years ago, the Georgia blue crab processors requested thermal processing assistance from the Marine Extension Service. The plant operators wanted to improve steam distribution within their retorts to provide for a more even cook. Poor steam distribution overcooks some crabs while undercooking others. The processors also wished to standardize their pasteurization operations, introduce product sizes different than the traditional 16 ounce can, and improve product cooling.

METHODS

In response to the processors' requests, the Marine Extension Service developed a portable system to monitor in-plant time/temperature relationships for the retorting of live blue crabs and the pasteurization of blue crab meat. A Newport Laboratories' 267B digital pyrometer capable of monitoring 10 separate thermocouples was interfaced with a Hewlett-Packard 9815A desk top computer through a Hewlett-Packard 9813AA BCD interface (Figure 1). The computer stored temperature data on a magnetic tape casette for later analysis. Temperature results were then plotted graphically against time using a Hewlett-Packard 7225A plotter. Internal crab and one pound (#401) can temperatures were monitored by 2-1/16" molded plastic Type T copper-constan thermocouples (O.F. Ecklund Company). Eight ounce (#307) can temperatures were monitored by 1-11/16" molded plastic Type T copper-constan thermocouples (O.F. Ecklund Company). A copper-constan thermocouple was inserted between the joint of the blue crab's swimmeret into the center of the body cavity and wired in place across the lateral spines with nickel-chromium wire. During most retort studies, two probes monitored steam temperatures at the top and bottom of the vertical retorts, and 8 probes monitored the internal temperature of crabs distributed throughout the cooking baskets. Temperature readings were taken electronically every 20 seconds. Thermocouples were inserted into the side of the cans to be pasteurized through a threaded stainless steel thermocouple receptacle. The receptacle was held in place through a hole previously cut by a can punch. The thermocouple, at the can's geometric center, was then sealed into the can with the appropriate amount of crab meat (Figure 2). Temperature values were carried electronically to the pyroscan through teflon coated copper-constan thermocouple wire. One probe monitored the water bath temperature and 9 probes monitored internal can temperatures with time. Temperature readings were taken once every minute during the cook.

Five examples of actual in-plant processing studies carried out at two plants are presented to show the capability of the system, 2 dealing

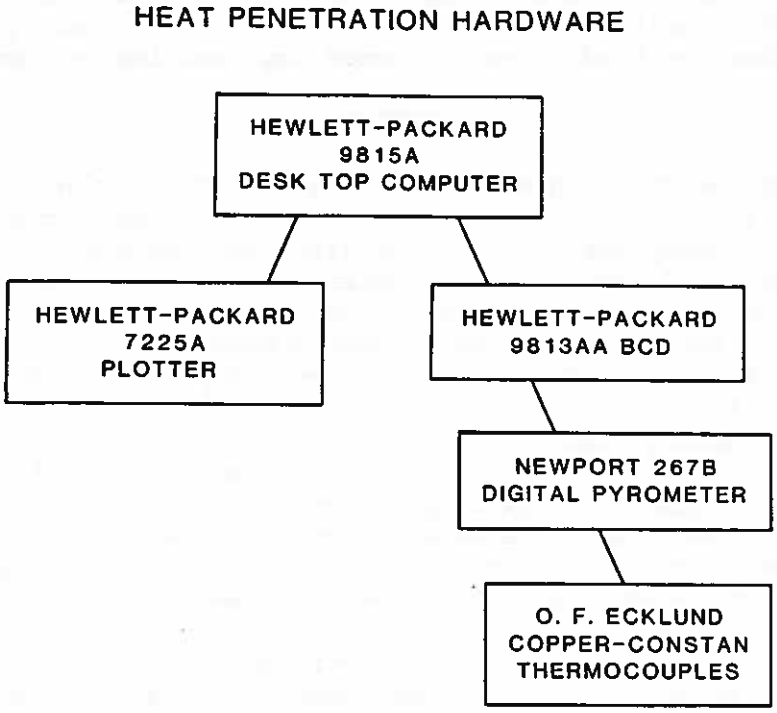
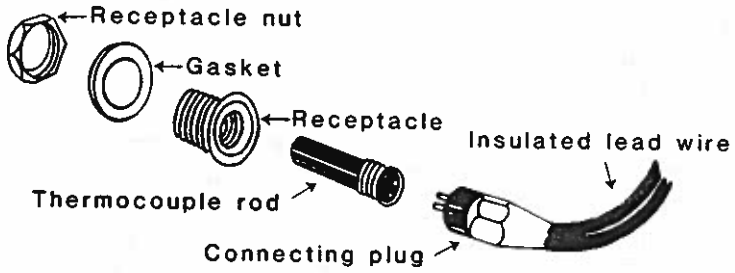
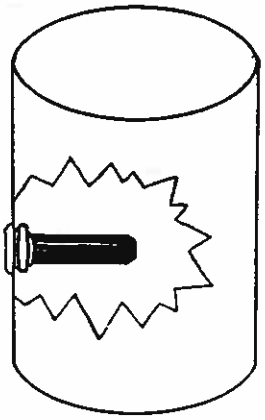


Figure 1. Heat penetration hardware used to record and output time/temperature processing data from blue crab processing plants.





Thermocouple at Geometric Center of Can

Figure 2. Detail of thermocouple placement in cans of crab meat to be monitored during pasteurization.

with retorted crabs, and three with pasteurized meat. In the first example the steam retort itself was modified to produce a more even cook, and in the second a new style crab retorting basket was compared with the baskets currently used by the processor. The pasteurization studies involved the adjustment of heating and cooling curves for 16 ounce cans of meat and the initial monitoring of an 8 ounce cook; the monitoring of 16 ounce can cooling rates were determined for a walk-in cooler after packing the cans in boxes for later shipment.

The results of the retort studies were interpreted graphically to determine maximum and minimum temperatures achieved by the 8 monitored crabs, the top steam time [time for the two open probes to reach maximum steam temperature, between 245° - 250°F (118.3° - 121.1°C)], and the evenness of the cook. Pasteurization studies were interpreted graphically to determine maximum internal can temperatures and evenness of the cooks. Additionally, F-values were determined for each of the 9 monitored pasteurized meat cans. Interval and cumulative F-values were calculated by the computer with the following formula (4):

$$F_0 = (t_2 - t_1)\left[10\,\frac{T_{1,2} - T_{RT}}{Z}\right]$$

F_0 = F-value of time internal $(t_2 - t_1)$

t_1 = Time of the first temperature measurement

t_2 = Time of the second temperature measurement

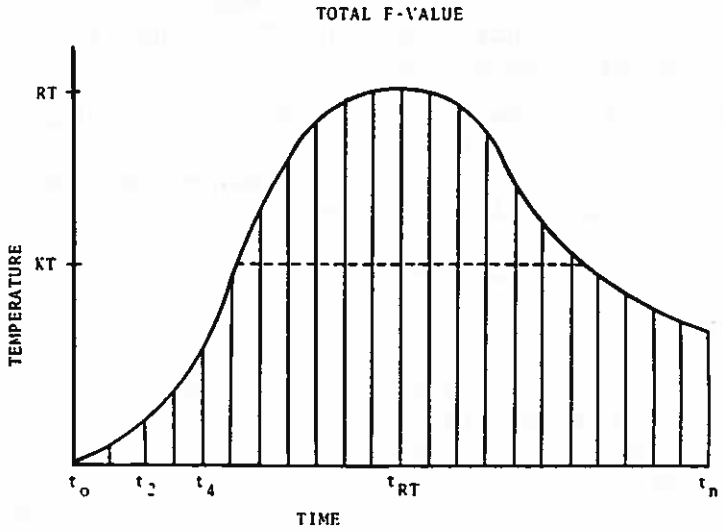
$T_{1,2}$ = Mean temperature in the internal $(t_2 - t_1)$

T_{RT} = Reference Temperature

Z = Z-value

Cumulative F-values were determined by summing internal F_0-values over time (Figure 3). The F-value is defined as the equivalent in minutes at a given temperature (T_{RT} = reference temperature) of all heat considered with respect to its capacity to destroy spores or vegetative cells of a particular organism (4, 5). The F-value is the number of minutes at a given temperature required to kill a known population of organisms in a given food under specified conditions. The traditional 16 oz (401) can of crab meat held at 185°F for one minute has an F-value of:

$$F_{185}^{16} = 24.47 \text{ minutes}$$



RT = Reference Temperature

KT = Killing Temperature

$$F_T = \sum_{o}^{n} F_o + F_1 + F_2 \cdots F_n$$

$$F_o = (t_2 - t_1)\ 10^{\frac{T_{1,2} - T_{RT}}{z}}$$

Figure 3. Summing the total F-value over time (t_o - t_n) for the intervals t_n - t_{n-1}.

If the can of crab meat could be instantly heated to 185°F, held at that temperature for 24.47 minutes and then instantly cooled, the total F-value would be 24.47 minutes. The traditional pasteurization process achieves the same equivalent F-value by heating and cooling the meat gradually over approximately a two to three hour period and holding the internal can temperature at 185°F for one minute (5). The superscript 16 (F^{16}_{185}) is the z-value determined for Clostridium botulinum type E. The z-value is defined as the number of degrees Fahrenheit required for the thermal destruction curve to traverse one log cycle. That is, a change in 16°F is required to reduce the number of bacterial vegetative and/or spore cells by a factor of ten. The higher the z-value, the greater the organism's resistance to heat.

Temperatures, F-values, and z-values are presented in terms of degrees Fahrenheit instead of degrees Celsius. The project was designed to directly assist the crab meat processors, who are not familiar with or comfortable with the Celsius system.

RESULTS AND DISCUSSION

A. Steam Retort Modification

On 9 June 1980, the operators of crab plant #1 requested assistance to improve the evenness of the cook for their crab retort. Approximately 1500 pounds (682 Kg) of live crabs were cooked in a vertical retort holding two large stainless steel cooking baskets (Figure 4). Eight thermocouples were inserted in live blue crabs. The crabs were then distributed through the two cooking baskets. Two open probes recorded steam temperature at the top and bottom of the retort (Figure 4). Figures 5 and 6 show the heat penetration curves determined during the cook. The initial cook was uneven with poor heat distribution throughout the retort. The processor increased the size of the steam inlet lines, added an additional steam inlet port, installed steam spreaders, and attached bleeder valves to the retort. We returned on 19 August 1980 to monitor the renovated retort. The results of the heat penetration data are presented in Figures 7 and 8. Probe number 6 failed to function during the processing run. The graphical data indicated a much more even cook than was achieved after the modifications were completed. Table 1 compares the two retort cooks. On 9 June 1980, crabs 4, 6, and 8 failed to reach 235°F obtaining 229°F, 208°F, and 225°F, respectively. The top steam time was 8 minutes. On 19 August 1980, one crab (#4) failed to reach 235°F. A maximum temperature of 232°F was achieved with a top steam time of 8 minutes. Come-up time was reduced from 6 to 5 minutes.

B. Testing A New Retort Basket Design

Specialists from the Marine Extension Service and the Cooperative Extension Service suggested design changes in the stainless steel retorting baskets used at crab plant #2. The operator built two new baskets,

Table 1. Summary of thermocouple retort studies, crab plant # 1, east retort, 9 June and 19 August 1980.

I. Come-up time for open probes

Probe #	9 June 1980		19 August 1980	
	Time	Temp	Time	Temp
1	6.0 min	250°F	5.0 min	250°F
10	-------	250°F	4.8 min	250°F

II. Probes within crabs that failed to Reach 235°F

Probe #	9 June 1980 Maximum Temperature	Probe #	19 August 1980 Maximum Temperature
4	229°F	4	232°F
6	208°F		
8	225°F		

III. Top steam time

9 August 1980	19 August 1980
8 min	8 min

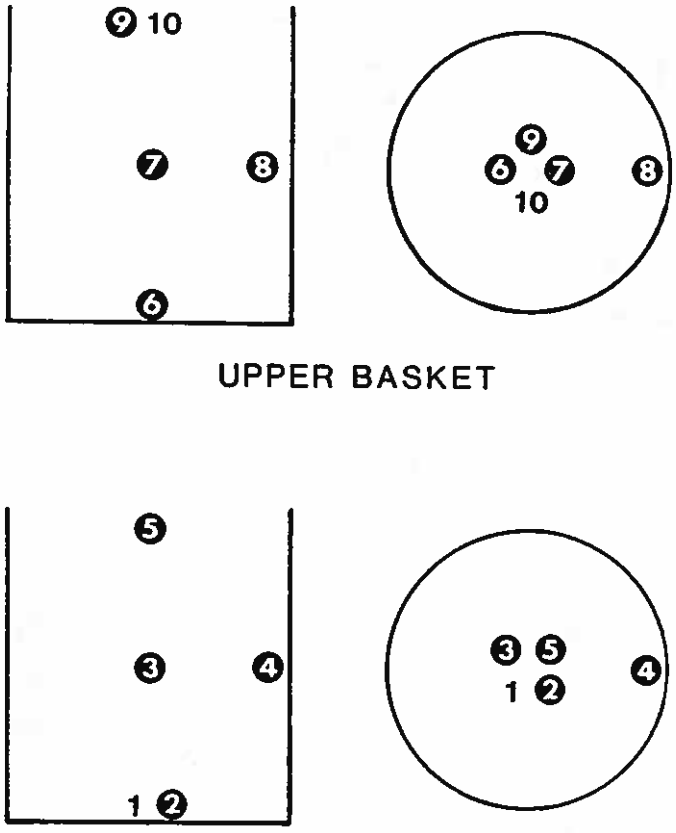


Figure 4. Placement of monitored blue crabs and open thermocouples in the retort baskets for all retort heat penetration studies.

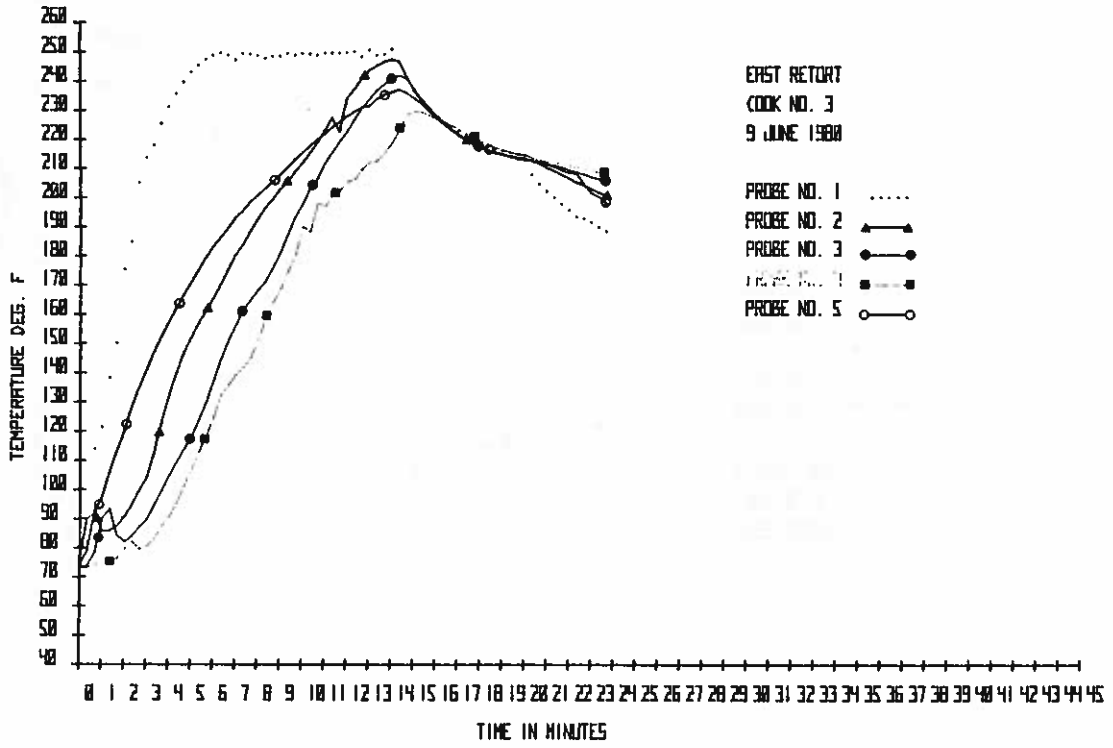


Figure 5. Thermal processing curves of steam retort on 9 June 1980 at crab plant # 1 prior to renovation. Probe # 1 monitored steam temperatures, probes # 2 - 5 measured internal crab temperatures.

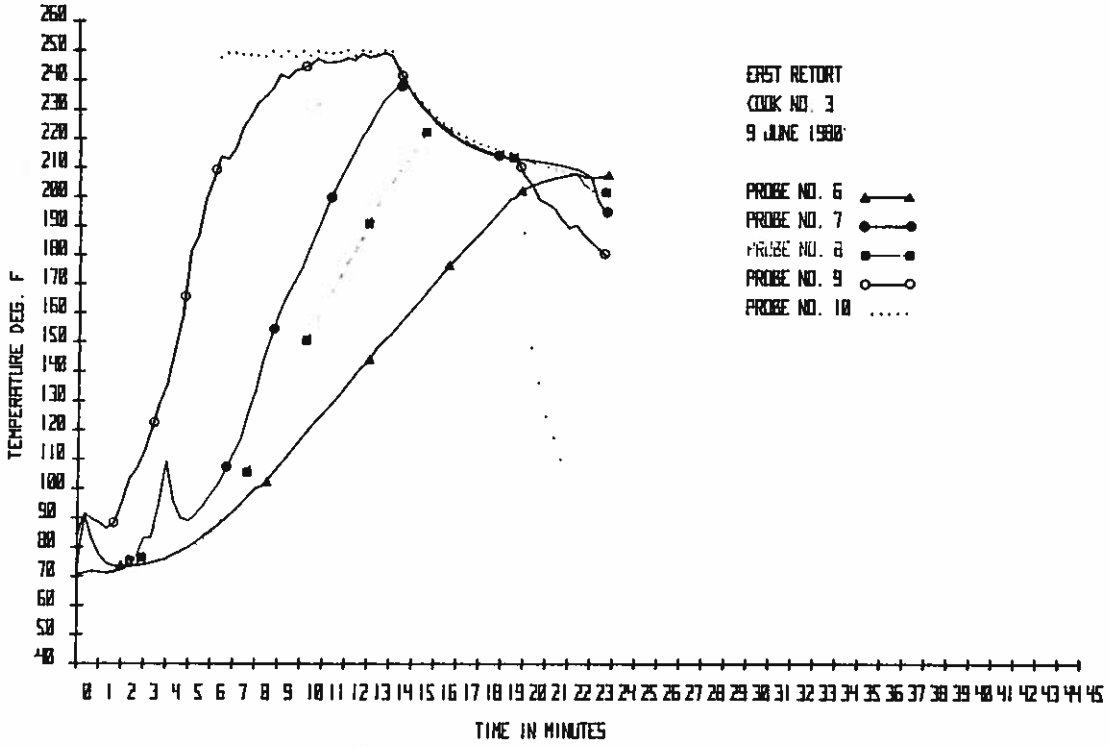


Figure 6. Thermal processing curves of steam retort on 9 June 1980 at crab plant # 1 prior to renovation. Probe # 10 monitored steam temperatures, probes # 6 - 9 measured internal crab temperatures.

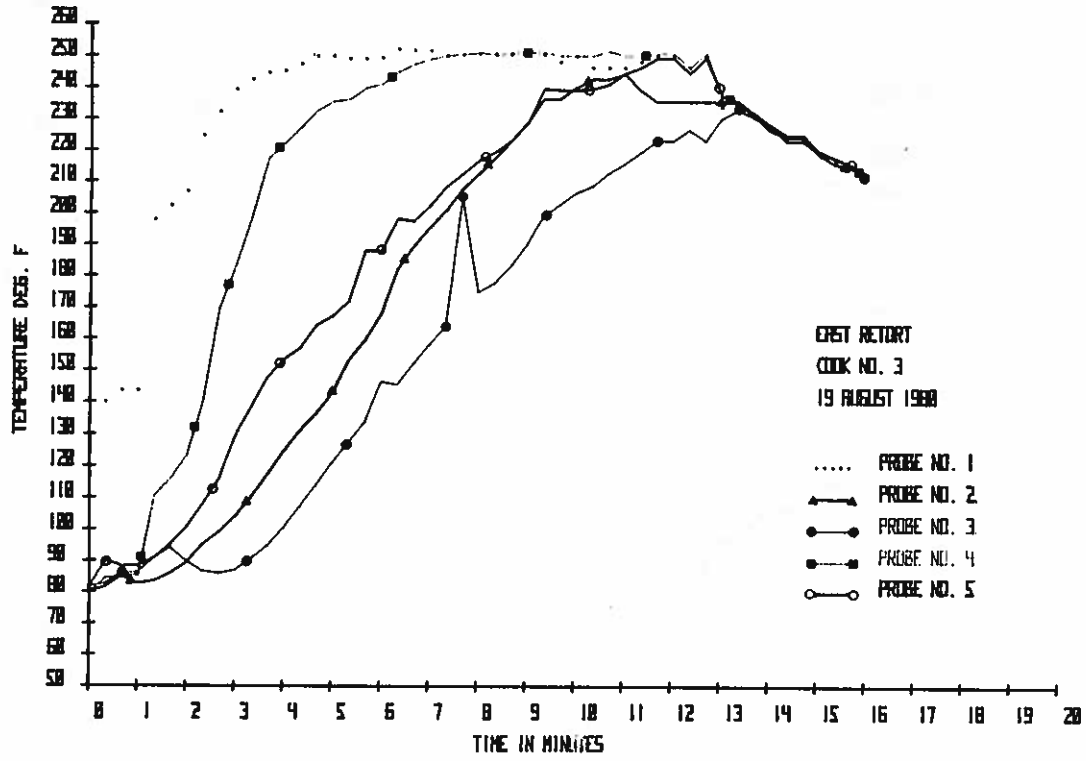


Figure 7. Thermal processing curves of steam retort on 19 August 1980 at crab plant # 1 after renovation. Probe # 1 monitored steam temperatures, probes # 2 - 5 measured internal crab temperatures.

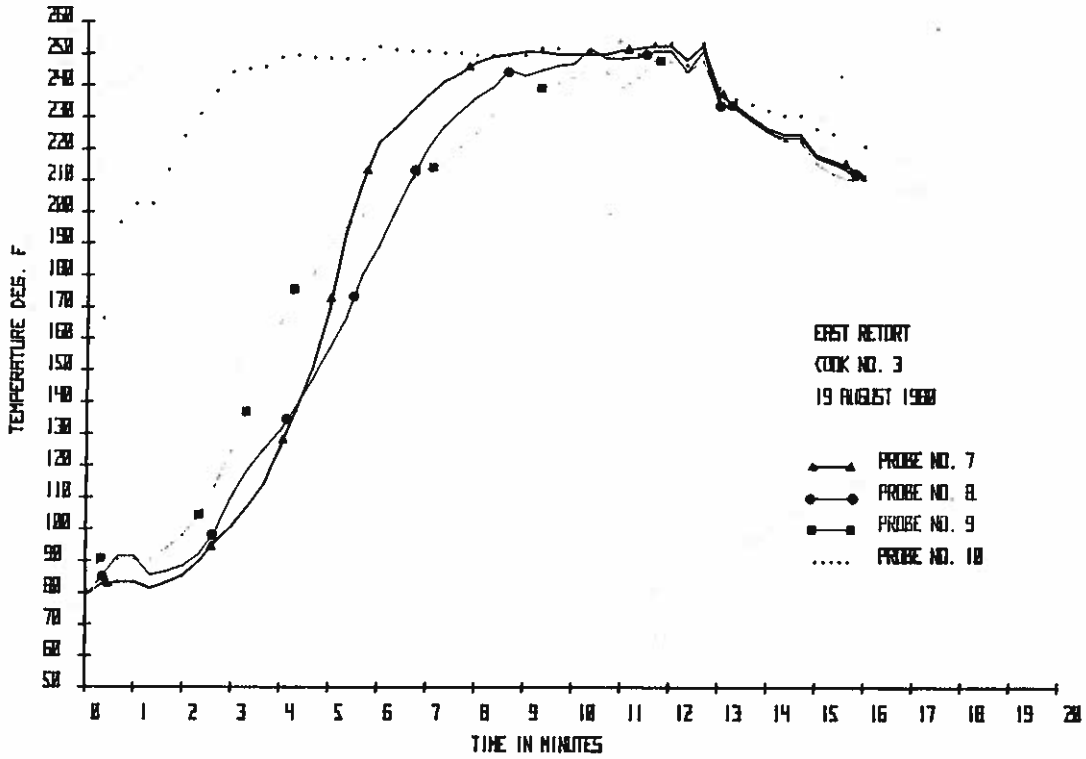


Figure 8. Thermal processing curves of steam retort on 19 August 1980 at crab plant # 1 after renovation. Probe # 10 monitored steam temperatures while probes # 7 - 9 measured internal crab temperatures.

each with perforated bottom doors and a perforated central triangular shaft that allowed more complete steam penetration throughout the basket (Figure 9). Both types of baskets could hold 500 - 800 pounds (227 - 364 Kg) of live crabs. Probes were placed within crabs and distributed throughout the baskets as in Figure 4. Figures 10 and 11 present the heat penetration curves from the retort utilizing the old style baskets (23 June 1983) and Figures 12 and 13 present the heat penetration curves for the new baskets (27 October 1982). The new baskets produced a more even cook and decreased the top steam time by 3 minutes (Table 2). The time interval required for the first and last crabs to reach 235°F after top steam time was achieved was 11:20 minutes for the old baskets and 7:10 minutes for the new baskets. Two crabs failed to reach 235°F in the old baskets (229°F and 233°F) and one crab failed to reach 235°F in the new baskets (232.5°F) (Table 3).

C. Pasteurization and Cooling of 16 Ounce Cans

On 11 August 1983, the pasteurization of a batch of 16 ounce crab meat cans was monitored. Figure 14 shows the distribution of the cans containing thermocouples throughout the water bath. Probes 1 - 9 monitored crab meat temperatures while probe #10 relayed water temperatures that ranged between 187.5 and 189.5°F throughout the cook. The pasteurization curves in Figures 15 and 16 indicated an even cook with a good cooling cycle after the water bath was drained and filled with an ice slurry. Maximum and minimum determined F-values were 21.63 and 30.73 (Table 4). The cans were held in the hot water bath for 102 minutes (Table 4). The operator wished to increase the F-value from 21.63 to a minumum level of 24.47, while decreasing the water bath temperature by 2°F to help prevent blueing of the meat. On 25 August 1983 a 16 ounce pasteurization process was recorded for a water bath that ranged between 185.7 - 187.3°F. The probed cans were distributed according to Figure 17, probes 1-8 recorded meat temperatures, probe #9 recorded water temperature. Again Figures 18 and 19 pointed to an even cook and a rapid cooling process. The cooking period was 21 minutes longer than the 11 August processing run, producing maximum and minimum F-values of 32.8 and 39.9. Each was well above the 24.47 minumum (Table 4).

Following 172 minutes in a circulating ice slurry, the 11 August 1983 pasteurized crab meat cans reached minimum and maximum temperatures of 35.78 and 38.48°F. On the 25 of August the cans were allowed to cool for 147 minutes to minimum and maximum values of 38.66 and 39.56°F. The current National Blue Crab Industry Association Standard for cooling requires crab meat to reach 36°F within 24 hours of pasteurization. Following the 25 August 1983 pasteurization of 16 ounce cans, the plant operator asked that the can temperatures be monitored after they were packed into wooden shipping boxes and placed in a large walk-in storage cooler. The cans were placed into three wooded shipping boxes and distributed according to Figure 20. The boxes were stacked in the cooler. Box number one was placed at the bottom of the stack of boxes, box number

Table 2. Summary of thermocouple retort studies, crab plant # 2, 23 June (old style baskets) and 27 October 1983 (new style baskets).

I. Come-up time for open probes

Probe #	23 June 1983		27 October 1983	
	Time	Temp	Time	Temp
1	12:20	245°F	12:30	245°F
10	12:30	245°F	12:30	245°F

II. Probes within crabs that failed to reach 235°F

Probe #	23 June 1983 Maximum Temperature	Probe #	27 October 1983 Maximum Temperature
2	233°F	3	233.5°F
6	229°F		

III. Top steam time

23 June 1983	27 October 1983
12 min	9 min

Date	Probe #	Minutes after top cook began to reach 235°F	Range In Minutes
6/23/83	9	2:40	
	8	4:20	
	5	4:40	
	4	5:00	
	7	5:20	
	3	11:40	
	2	13:20[1]	
	6	14:00[2]	11:20
10/27/83	9	2:50	
	2	4:10	
	7	5:00	
	6	5:30	
	8	6:10	
	5	8:00	
	4	9:10	
	3	10:00[3]	7:10

[1] reached 233°F

[2] reached 229°F

[3] reached 232.5°F

Table 3. Comparison of time required for blue crabs to reach 235°F for the old and new style baskets at crab plant # 2.

Table 4. Heat penetration data from 16 ounce pasteurization studies at crab plant # 2 on 11 August and 25 August 1983.

Date	11 Aug 1983	11 Aug 1983	25 Aug 1983	25 Aug 1983
Probe Number	8	2	5	4
Relative Temperature	Coldest	Hottest	Coldest	Hottest
Minutes in Water Bath	102	102	123	123
Maximum Heat Temperature	182.66°F	185.18°F	184.1°F	185.2°F
Maximum F-Value	21.63	30.73	32.8	39.9
Minutes From Immersion to reach F = 24.5	-----	107	127	119
Water Temperature	187.5-189.5°F	187.5-189.5°F	185.7-187.3°F	185.7-187.3°F

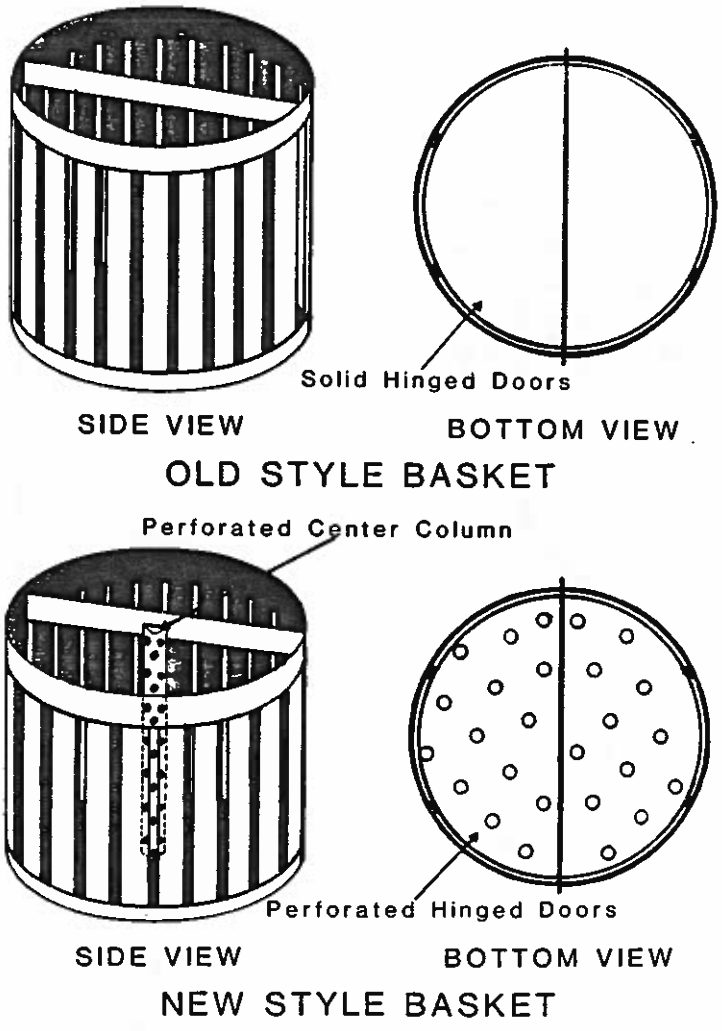


Figure 9. Comparison of old and new style crab retort baskets used at crab plant # 2.

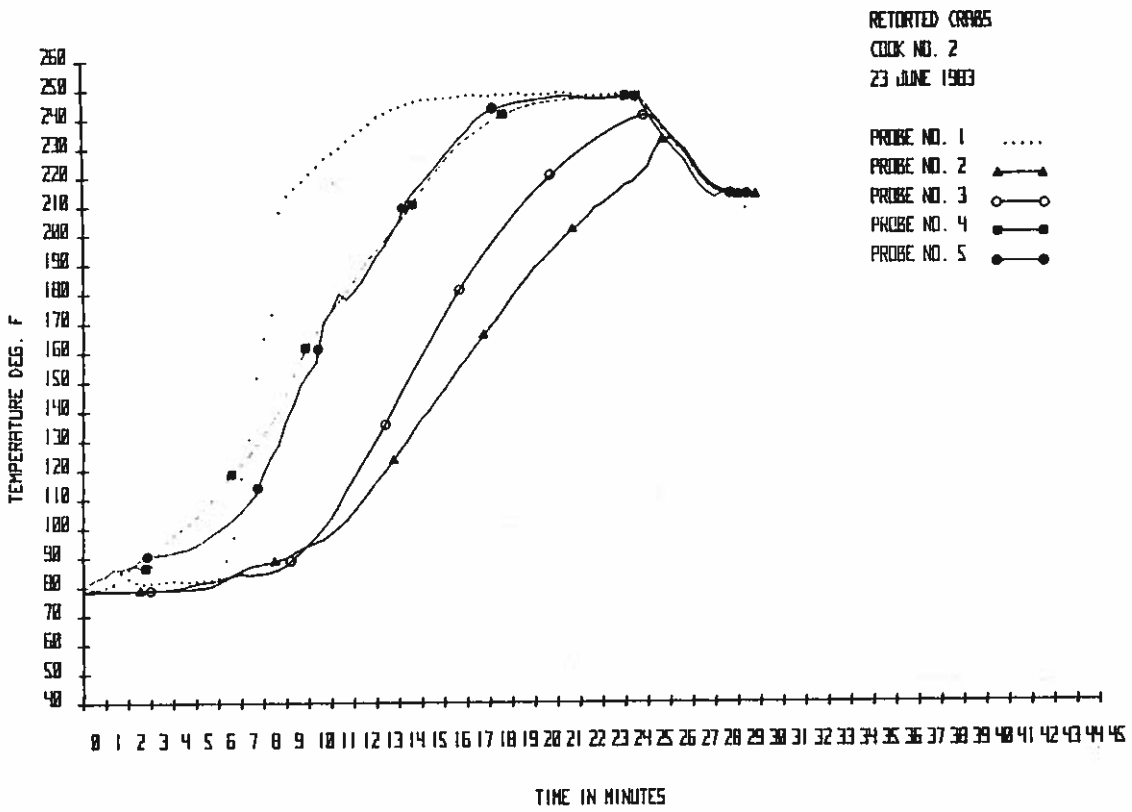


Figure 10. Thermal processing curves of the steam retort at crab plant # 2 on 23 June 1983 utilizing the old sytle retort basket. Probe # 1 monitored steam temperatures while probes # 2 - 5 measured internal crab temperatures.

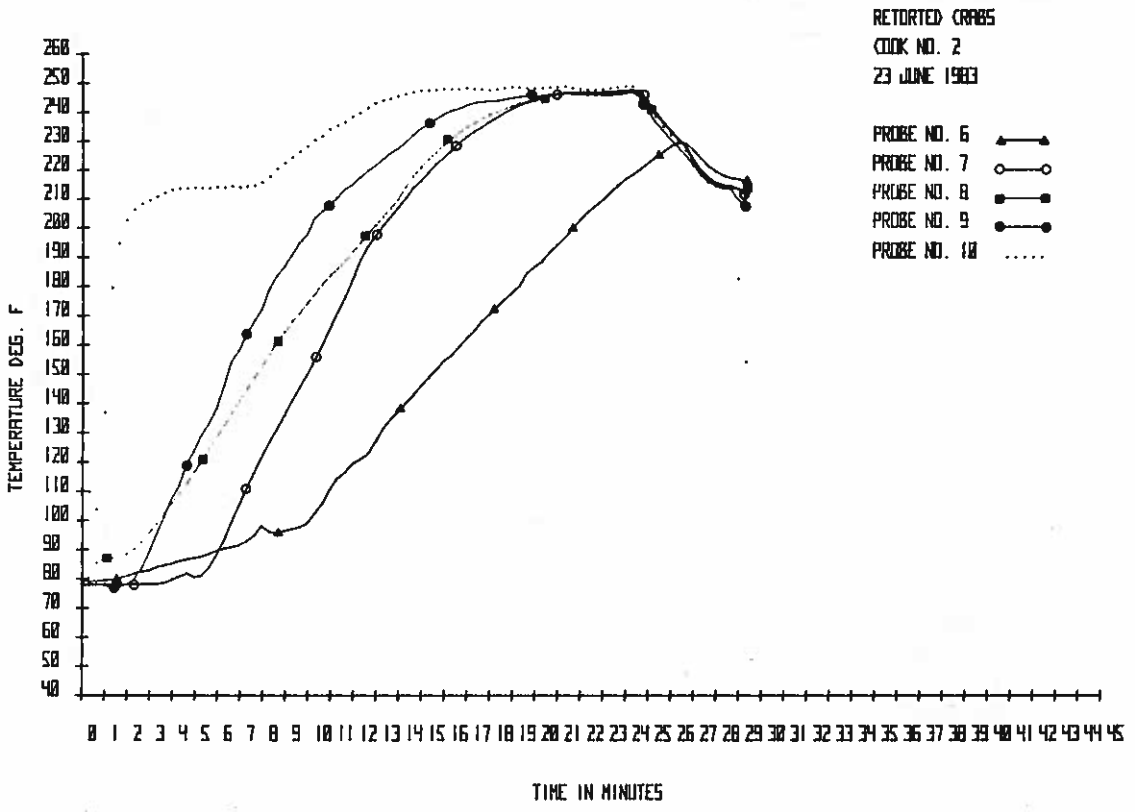


Figure 11. Thermal processing curves of the steam retort at crab plant # 2 on 23 June 1983 utilizing the old style retort baskets. Probe # 10 monitored steam temperatures while probes # 6 - 9 measured internal crab temperatures.

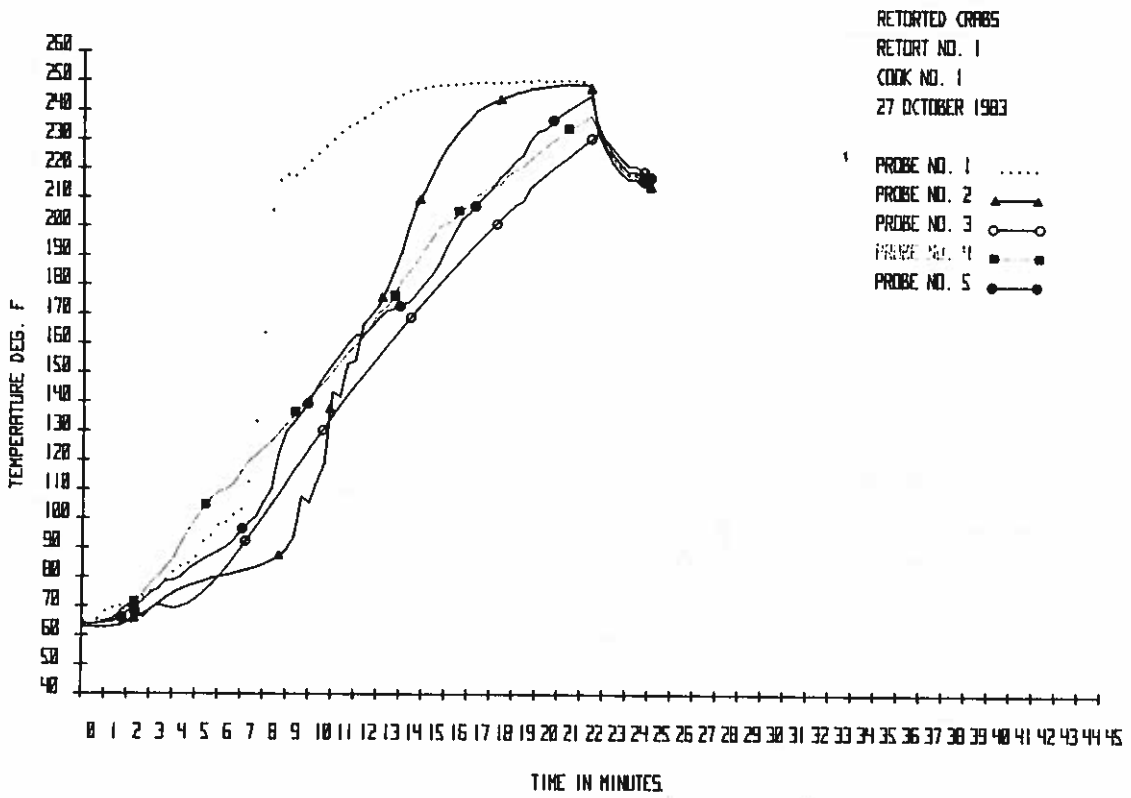


Figure 12. Thermal processing curves of the steam retort at crab plant # 2 on 27 October 1983 utilizing the new style retort baskets. Probe # 1 monitored steam temperature while probes # 2 - 5 measured internal crab temperatures.

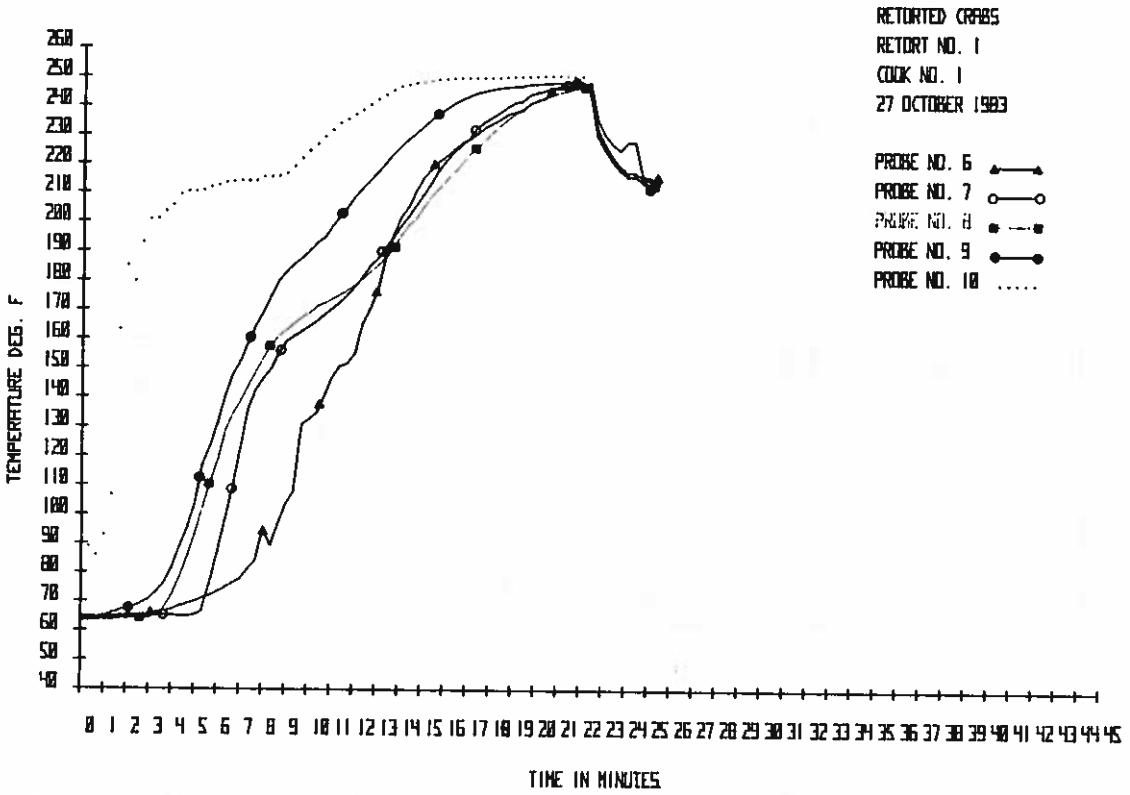
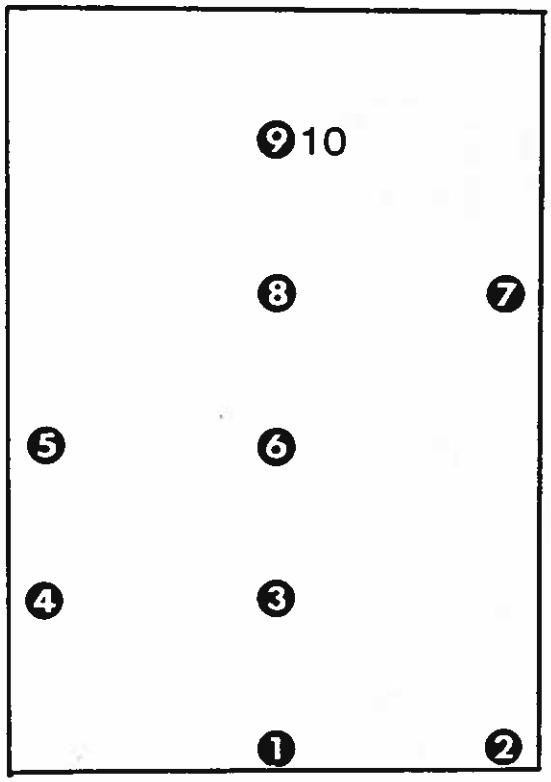


Figure 13. Thermal processing curves of the steam retort at crab plant # 2 on 27 October 1983 utilizing the new style retort baskets. Probe # 10 monitored steam temperatures while probes # 6 - 9 measured internal crab temperatures.

16 oz. Pasteurization

11 August 1983



Pasteurizing Basket

Figure 14. Placement of the thermocouples in the water bath at plant # 2 on 11 August 1983. Probes # 1 - 9 monitored the internal temperature of 16 ounce cans of crab meat during pasteurization. Probe # 10 measured water temperatures.

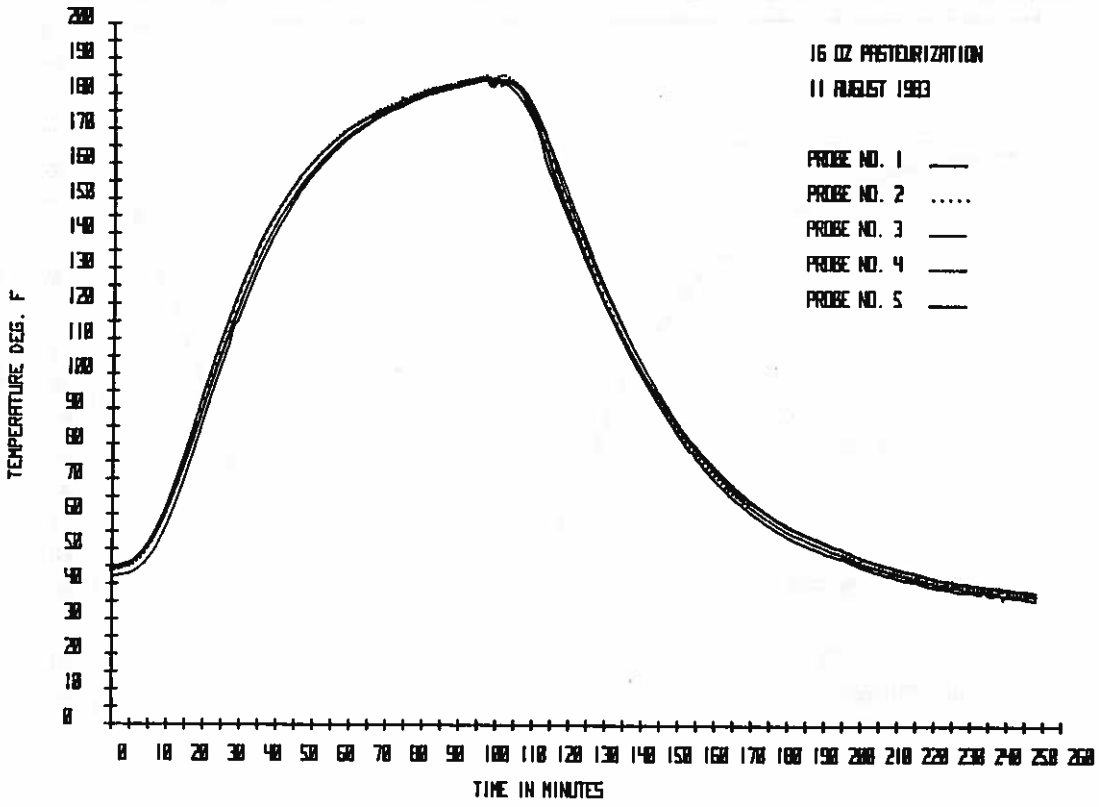


Figure 15. Thermal processing curves for 16 ounce pasteurized crab meat cooked on 11 August 1983 at plant # 2. Probes # 1 - 5 measured the internal temperatures of crab meat.

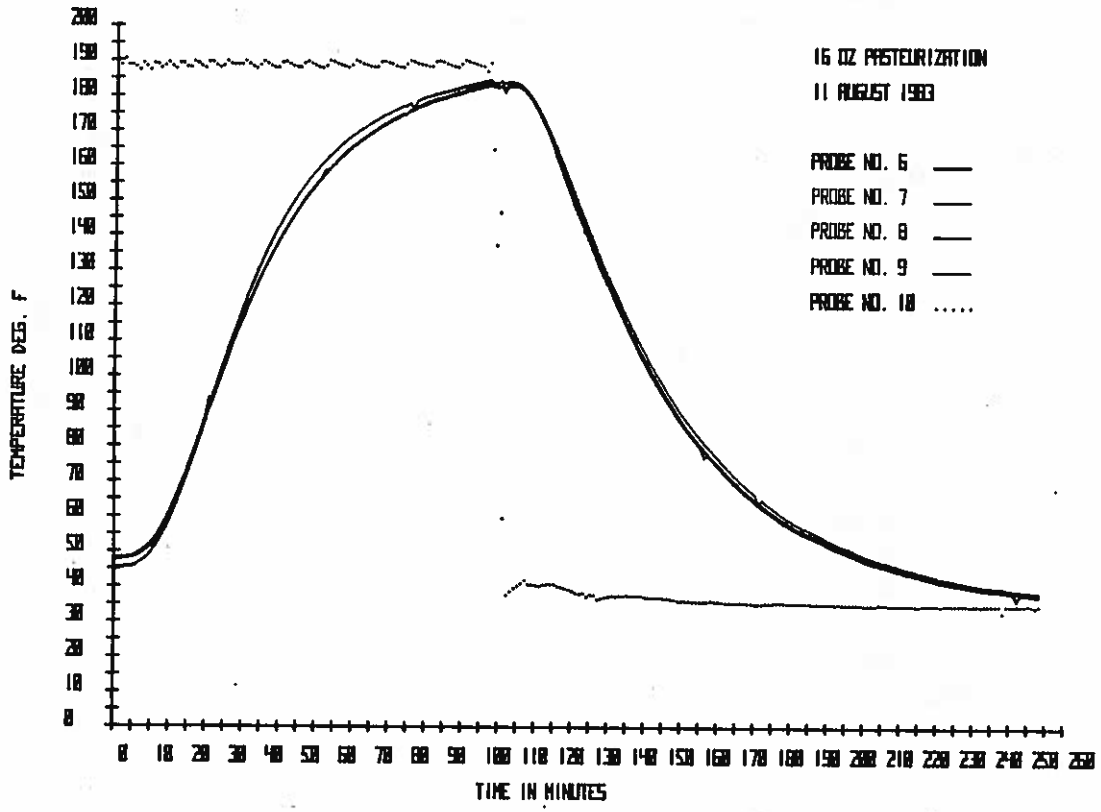
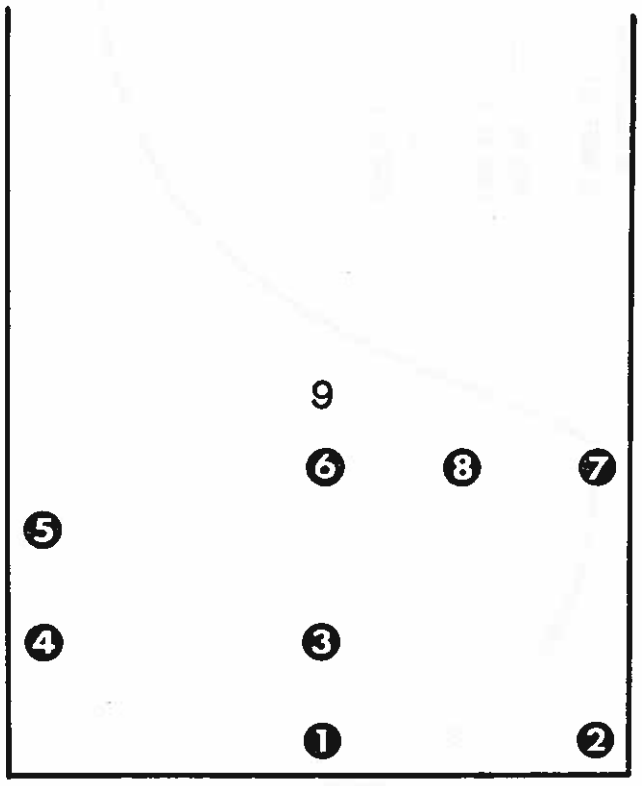


Figure 16. Thermal processing curves for 16 ounce pasteurized crab meat cooked on 11 August 1983 at plant # 2. Probes # 6 - 9 measured the internal temperature of crab meat while probe # 10 monitored water temperature.

16 oz. Pasteurization

25 August 1983



Pasteurizing Basket

Figure 17. Placement of thermocouples in the water bath at plant # 2 on 25 August 1983. Probes # 1 - 8 monitored the internal temperatures of 16 ounce cans of crab meat during pasteurization. Probe # 9 measured water temperatures.

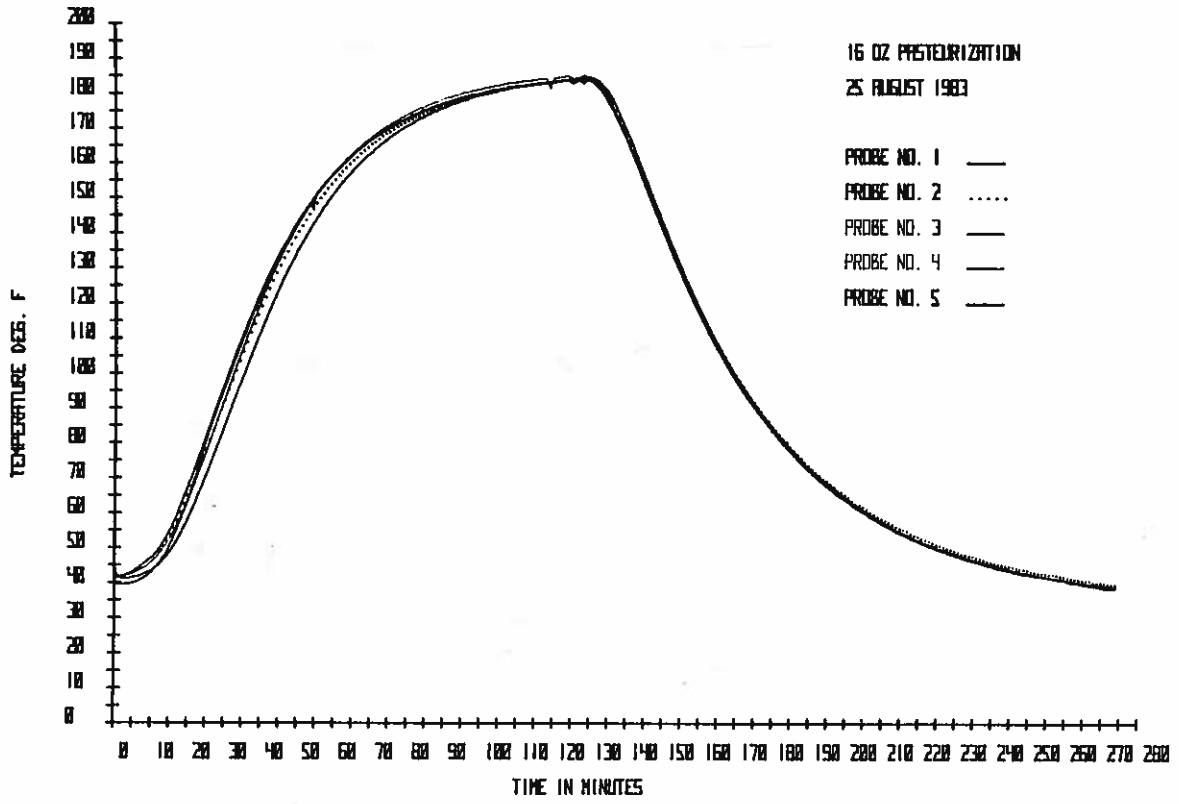


Figure 18. Thermal processing curves for 16 ounce pasteurized crab meat cooked on 25 August 1983 at plant # 2. Probes # 1 - 5 measured the internal temperatures of crab meat.

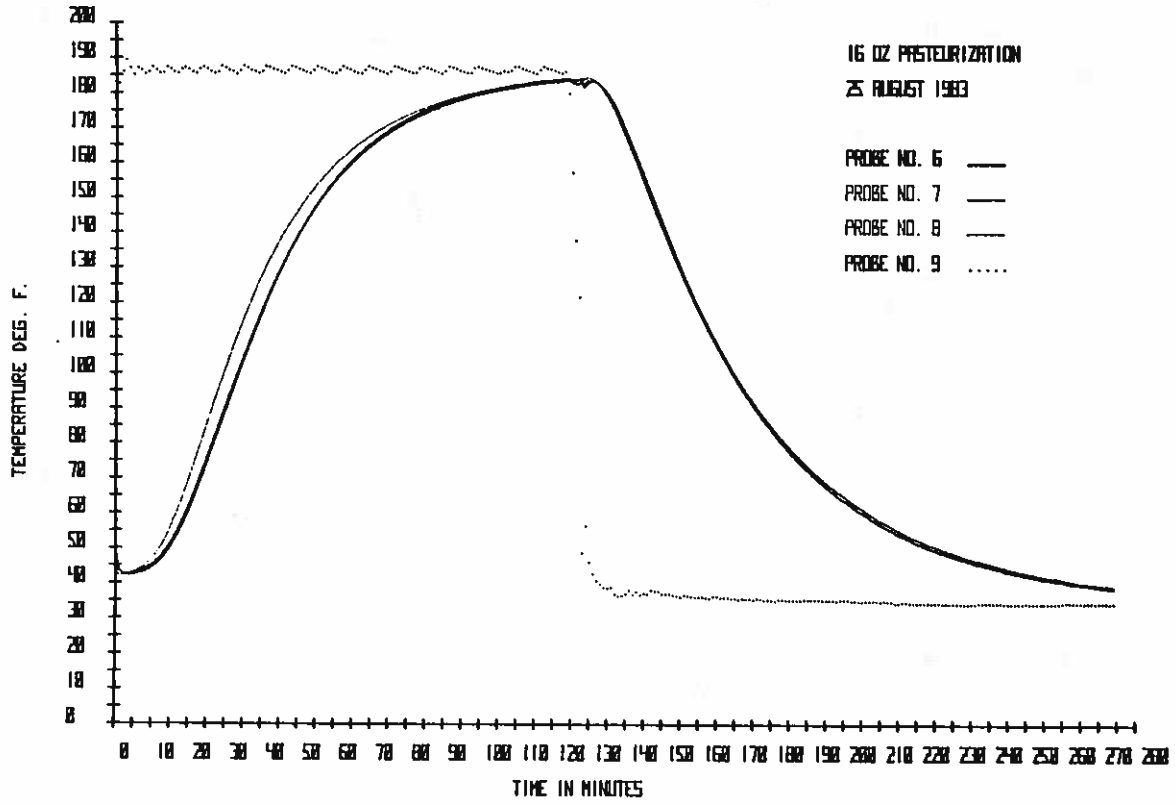


Figure 19. Thermal processing curves for 16 ounce pasteurized crab meat cooked on 25 August 1983 at plant # 2. Probes # 6 - 9 measured the internal temperatures of crab meat while probe # 10 moniotred water temperatures.

two in the center, and box number three on top. Probes 1-8 recorded meat temperatures while probe #9 recorded air temperatures. The cooler temperature ranged between 32 and 39.92°F through 92 hours of storage, however, the temperature remained below 36°F the majority of the time (Figure 21). All stored cans reached 36°F within 14 hours.

D. Pasteurization of 8 Ounce Cans

The operators of plant #2 pasteurized a test batch of crab meat packed in 8 ounce cans on 28 July 1983. Nine thermocouple probes, 1 - 9, were placed in cans of meat and sealed. The cans were distributed through the pasteurization basket according to Figure 22. Probe #10 monitored the water temperature of the bath. The cans were held in hot water that ranged between 187.34 and 190.04°F for 65 minutes. The 8 ounce pasteurization produced an even cook, however, an F-value = 24.48 was not achieved by any of the monitored cans (Figures 23 and 24). The hottest probe, number 1, reached a maximum temperature of 180.5°F with a total F-value of 9.46. Probe number 8 reached the lowest temperature, 178.52°F, with a total F-value of 5.08. The product was grossly undercooked. Additional work with 8 ounce cans will be completed later this year.

CONCLUSIONS

The establishment of proper time/temperature heat penetration relationships during the processing of live blue crabs and pasteurized crab meat are vital to the safety, quality, and economic viability of products produced by blue crab processors. Most coastal crab plants are small operations that have neither the expertise nor the necessary equipment to determine proper time/temperature processing parameters. A desk top computer coupled to a pyroscan temperature recorder have provided an effective means to monitor time/temperature relationships in the field for retorted live crabs, and the pasteurization and cooling of crab meat packed in cans.

The Marine Extension Service has made the recording equipment and seafood specialists available to Georgia crab processors. To date, the portable field unit has been successfully used to direct the renovation of a large pressurized steam retort, providing a more even energy efficient cook of live blue crabs. The effectiveness of a newly designed retort basket was demonstrated to another crab processor. The quality and safety of pasteurized crab meat produced at one plant has been improved by adjusting water bath pasteurization temperatures and times, and by standardizing initial and final product cooling in an ice/water slurry and a walk-in cooler, respectively. Processing schedules have been defined in terms of F-values instead of the traditional cook which applied only to one pound 401 cans that were required to reach an internal temperature of 185°F (85°C) for one minute. The use of F-values have provided processors with greater flexibility. Meat can be safely

16 oz. Pasteurized Cans
Refrigerated Storage
25 August thru 29 August 1983

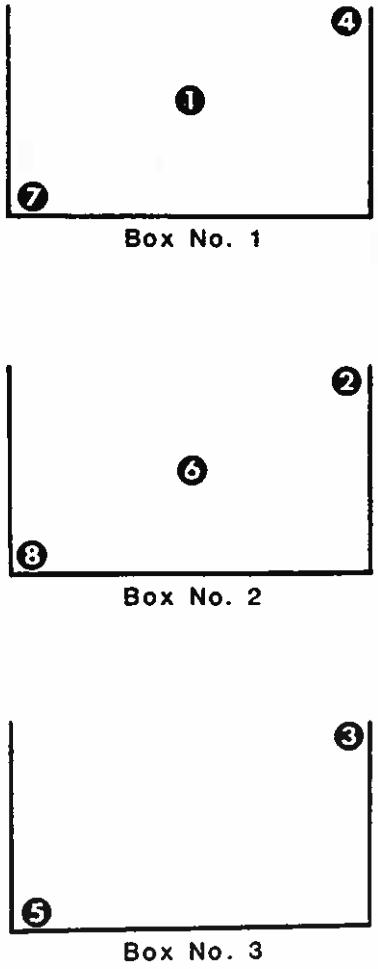


Figure 20. Placement of 16 ounce cans of pasteurized crab meat in wooden shipping cartons prior to monitoring temperature change with time while the meat was stored in a walk-in cooler at plant # 2.

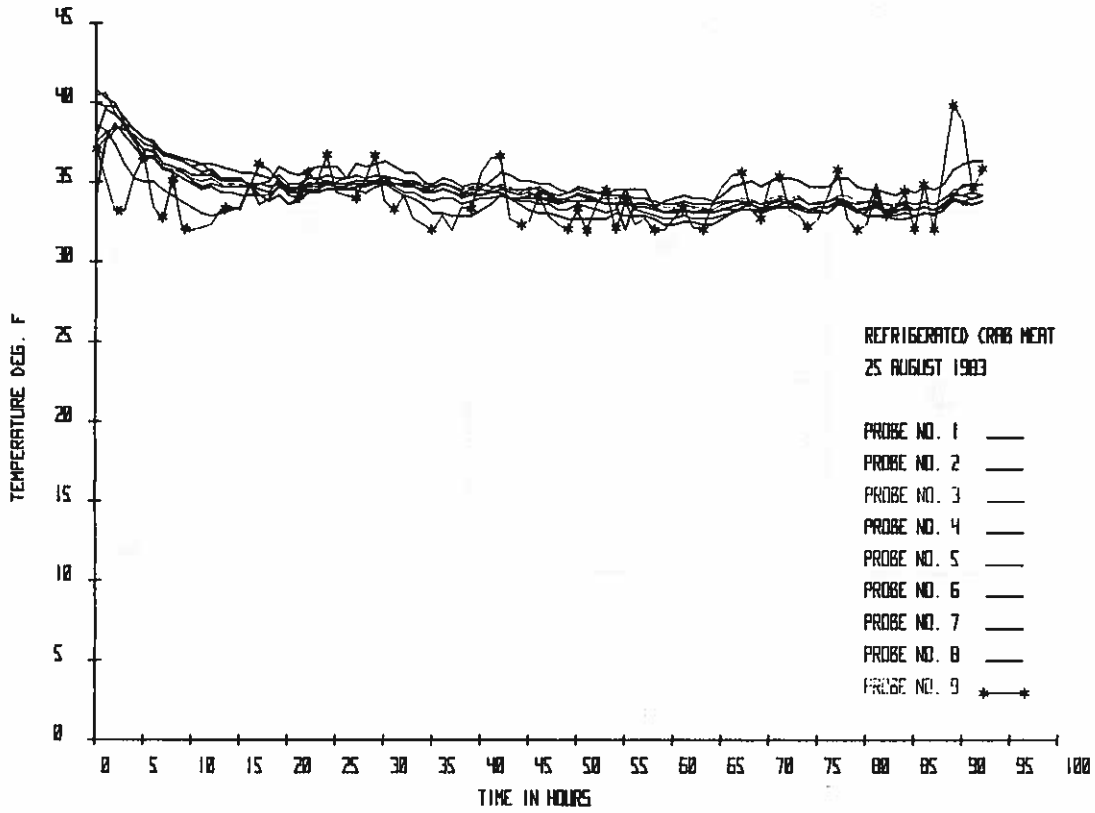
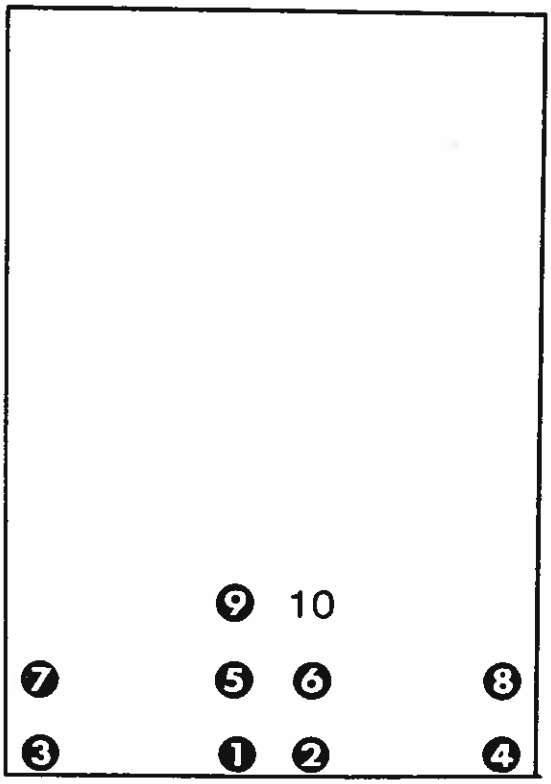


Figure 21. Monitored temperatures of 16 ounce crab meat cans stored in wooden shipping boxes and held in a walk-in cooler at plant # 2 on 25 August 1983 for 92 hours. Probes # 1 - 9 measured crab meat temperature while probe # 10 recorded air temperatures.

8 oz. Pasteurization

28 July 1983



Pasteurizing Basket

Figure 22. Placement of thermocouples in the water bath at plant # 2 on 28 July 1983. Probes # 1 - 9 monitored the internal temperatures of 8 ounce cans of crab meat during pasteurization. Probe # 10 measured water temperatures.

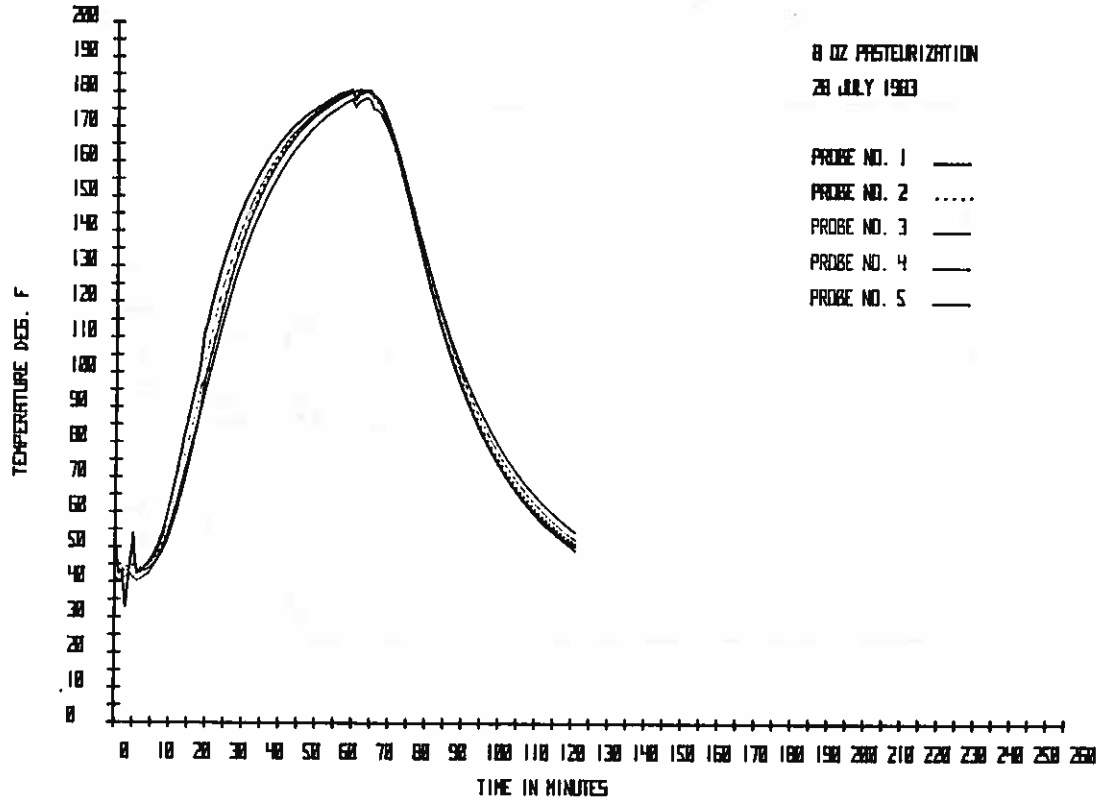


Figure 23. Thermal processing curves for 8 ounce pasteurized crab meat cooked on 28 July 1983 at plant # 2. Probes # 1 - 5 measured the internal temperatures of crab meat.

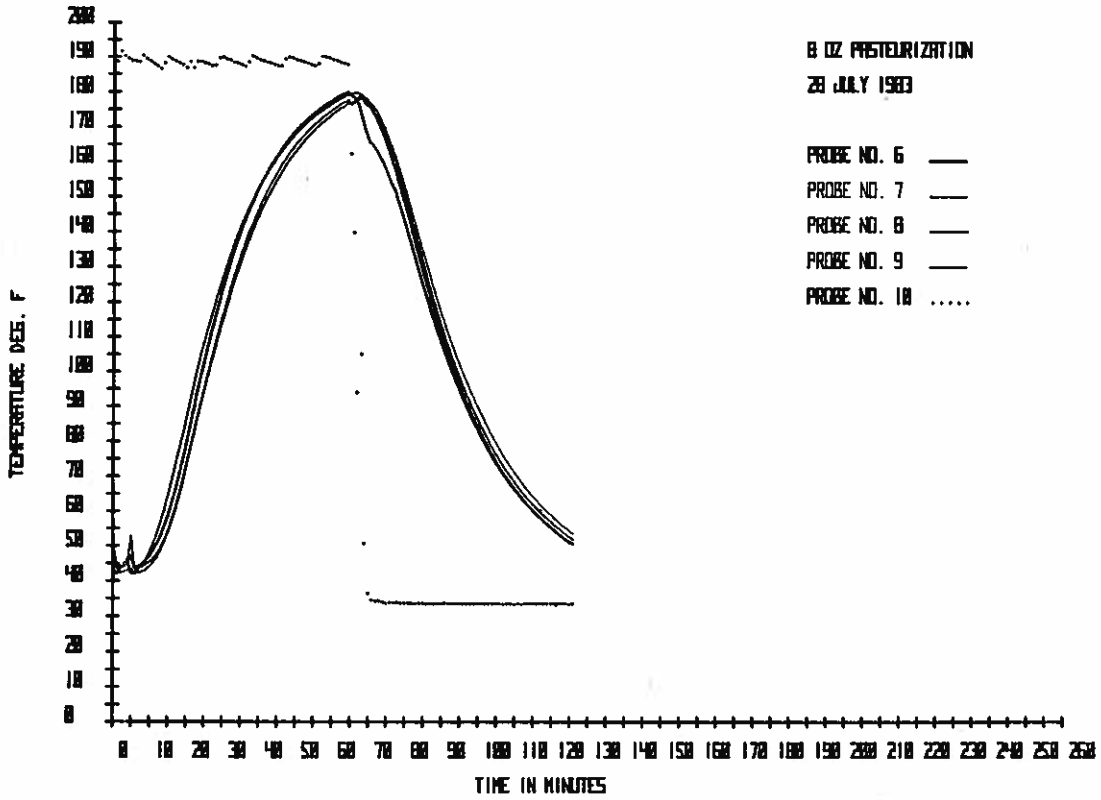


Figure 24. Thermal processing curves for 8 ounce pasteurized crab meat cooked on 28 July 1983 at plant # 2. Probes # 6 - 9 measures the internal temperatures of crab meat while probe # 10 monitored water temperatures.

pasteurized at lower water bath temperatures for longer periods, reducing the probability of blueing, particularly in South Atlantic and Gulf of Mexico crab meat which is more susceptible to discoloration. Finally, the standardization and documentation of crab cooking and pasteurization processes brings the plant owners and managers within compliance of the new National Blue Crab Industry Pasteurization Guidelines (1, 2, and 5).

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Jack Amason of Sea Gardens Seafood, Valona, Georgia and Mr. David Lewis of the Lewis Crab Factory, Brunswick, Georgia for their help and cooperation during this study. The technical assistance of Ms. Kathy Bennett is greatly appreciated.

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COMPARATIVE ANALYSIS OF SHRIMP BLOCK THAWING METHODS

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Net weights for block frozen shrimp were obtained by the official tank thawing method and by a newly developed method using a shower head spray. The average time of thawing per block was 44 minutes using the official method whereas the new method yielded an average thaw time of 23 minutes per block. A statistical comparison of the sample means from each method was performed using the two group t test. The observed agreement between the sample means and their nonsignificant difference suggests that the two methods should produce equivalent mean net weights.

INTRODUCTION

Over the past three years much controversy has been raised concerning the methods used to determine the net weight of frozen shrimp blocks. The method outlined in the thirteenth edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) (sections 18.015 and 18.016) has been used by the Food and Drug Administration, Fish and Wildlife Service and the United States Department of Commerce for many years. In May 1982, the Department of Commerce published an interim Standard for Grades of Fresh and Frozen Shrimp in the Federal Register. These standards included the official method for final action mentioned above.

The official method has proved highly reliable and the results have been consistently reproducible. However, at the quality control level where dozens of blocks must be thawed in the course of a net weight analysis, the amount of time and the tally of equipment costs can be very large. Therefore an alternative thawing method, combining the techniques described in sections 18.001, 18.015 and 18.016 of the AOAC manual, was used for the purposes of this experiment.

METHODS AND MATERIALS

Frozen raw shrimp blocks were randomly sampled from seven lots of raw, peeled, undeveined, imported Thailand shrimp. The blocks had declared net weights of 2 kilograms (4.4 pounds) each and were comprised of penaeid shrimp of the species Penaeus merguiensis and Penaeus esculentus. The sizes of the shrimp were between 40-50 and 110-130 counts per pound. Each block was unwrapped and weighed for a gross frozen weight.

Twenty-four blocks were thawed following the offiical method and thirty-three blocks were thawed using the modified, shower head spray method.

The official procedure reads as follows (CFR Title 50, 1982; AOAC, 1978):

A). Equipment needed

1. A container of sufficient size and capacity so as to completely submerge the product.
2. Wire mesh baskets large enough to hold contents. of 1 package and with openings small enough to retain all pieces.
3. A balance sensitive to 0.01 ounce or 0.025 grams.
4. A U.S. standard #8 wire sieve, 12 inch diameter.
5. A thermometer.
6. A stop watch.

B). Procedures

Place contents of individual package in wire mesh basket and immerse in container of fresh water so that top of basket extends above water level. Introduce water of $80^{\circ} \pm 5^{\circ}$ F ($26^{\circ} \pm 3^{\circ}$ C) at bottom of container at a flow rate of 1 - 3 gallons (3.8 - 11.4 liters) per minute. As soon as product thaws so that the glaze can be removed and the shrimp separate easily, transfer all material to a 12 inch (30 Centimeter) No. 8 sieve, distributing evenly. Tilt the sieve above 20 degrees and drain for exactly 2 minutes. Immediately transfer shrimp to a tared container and weigh.

Due to the fact that most quality control laboratories and production facilities have sinks available for use, an inexpensive conversion to a shower head spray was accomplished with little difficulty. The author converted an existing spigot with a shower head, two hose clamps and a 3 inch piece of garden hose for $6.64, in fifteen minutes. With this converted equipment the thawing procedure was as follows:

A). Equipment Needed

1. A sink with converted shower head spray.
2. A U.S. standard #8 sieve, 12 inch diameter.
3. A thermometer.
4. A stop watch.

B). Procedures

Place contents in sieve and place in sink. Introduce water of $80^{\circ} \pm 5^{\circ}$ ($26^{\circ} \pm 3^{\circ}$ C) at a flow rate of 1 to 3 gallons (3.8 - 11.4 liters) per minute through the shower head and allow water to drain from sink (method similar to AOAC section 18.001). Block can be turned several times during thawing. Frozen shrimp that are caked together may be parted manually provided they are not injured in the process. When all the ice has melted and the shrimp separate easily, shut water off and distribute the shrimp evenly in the sieve. Tilt the sieve to above 20 degrees and drain for exactly two minutes. Immediately transfer shrimp to a tared container and weigh.

RESULTS

The net weight for each block was recorded as it was analyzed, and the data given in Table I. A mean ($\bar{x}$), standard deviation(s) and standard error of the mean (SE) were calculated for each method's data. An average (mean) net weight of 4.25 lbs $\pm$ 0.02 lbs was determined for the twenty-four samples that were analyzed by the official method. Similarly, an average net weight of 4.23 lbs $\pm$ 0.01 lbs was calculated for the thirty-three samples that were analyzed by the spray method (see Tables I and II). To determine if the two sample averages are sufficiently different to support that the two methods actually produce different average net weights, the two group t test was performed resulting in a t score of 0.9452 with 55 degrees-of-freedom (see Table III and Reference 1).

DISCUSSION AND CONCLUSIONS

The two group t test is an appropriate technique to determine the probability of rejecting a true null hypothesis (no difference hypothesis) between two population means for the independent samples case. That is, this test will yield the probability (called the p-value) of observing a difference between the two sample means when, in fact, the population means are equal.

The resulting t score of 0.9452 with 55 degrees-of-freedom indicates that the sample means are statistically different at a level of significance (p-value) of 35%. That is, the data is sufficient to support that the two methods will yield different results provided a 35% chance of an incorrect decision is acceptable. However, when involved with a subject as sensitive as net weight analysis, a 35% chance of an incorrect decision is

unacceptable, especially when stating that the two methods yield different results. Furthermore, the t test does not show sufficient evidence to reject the no difference hypothesis. Therefore based on the data and appropriate analysis, the suggested conclusion is that the sample means are not in sufficient disagreement to support different mean net weight determinations between the two methods.

Additionally, and of certain practical significance is the time factor differential between the two methods. For the 33 blocks that were analyzed by the spray method, the average time of thawing was 23 minutes per block. Using the official method, however, the average thawing time was 44 minutes per block.

In light of this fact, a substantial savings of time, water and ultimately, money are important benefit of using the modified spray method.

Since the preliminary results show that the new method compares very favorably with the current official method and offers a significant time savings, further collaborative study should be initiated in order that the new method may some day be utilized for official purposes.

Table I

Official Method*		Spray Method*	
4.26	4.20	4.12	4.25
4.22	4.16	4.10	4.24
4.24	4.18	4.12	4.24
4.22	4.36	4.13	4.30
4.24	4.28	4.34	4.32
4.26	4.37	4.18	4.20
4.18	4.38	4.21	4.28
4.38	4.30	4.24	4.10
4.38	4.24	4.26	4.22
4.36	4.30	4.22	4.18
4.16	4.00	4.34	4.26
4.16	4.16	4.24	4.22
		4.24	4.24
		4.26	4.28
		4.34	4.29
		4.26	4.22
			4.22

n_1=24 (samples)

$\bar{x}_1$=4.25 lbs.

s_1=0.094 lbs.

$SE_1 = \frac{s_1}{\sqrt{n_1}}$=0.02 lbs.

*Net weight in pounds

n_2=33 (samples)

$\bar{x}_2$=4.23 lbs.

s_2=0.065 lbs.

SE_2=0.01 lbs.

Table II

$$SE_1 = \frac{s_1}{\sqrt{n_1}} = \frac{0.094}{\sqrt{24}} = 0.02$$

$$SE_2 = \frac{s_2}{\sqrt{n_2}} = \frac{0.065}{\sqrt{33}} = 0.01$$

Table III

t Test

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 \quad n_2 \quad - \quad 2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

$n_1 = 24 \qquad \bar{x}_1 = 4.25 \qquad s_1^2 = .0089$

$n_2 = 33 \qquad \bar{x}_2 = 4.23 \qquad s_2^2 = .0043$

$$t = \frac{4.25 - 4.23}{\sqrt{\frac{(23)(.0089)+(32)\ (.0043)}{24 \quad 33 - 2}\left(\frac{1}{24}+\frac{1}{33}\right)}} = \frac{.02}{\sqrt{\left(\frac{.3423}{55}\right)\quad\left(\frac{57}{792}\right)}}$$

$$t = \frac{.02}{\sqrt{.000448}} = \frac{.02}{.02116} = .9452 \text{ with 55 degrees of freedom}$$

p-value=35%

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Note: Special thanks are extended to Mr. John Allen, whose assistance and cooperation are greatly appreciated.

Developing Vessel Level Grade Quality Standards for the Shrimp Industry

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INTRODUCTION

The shrimp industry is in need of a fast, accurate, simple and economic method to evaluate the quality of shrimp. If such a method could be proposed and successfully implemented, the catches could be discriminated into quality groups or categories at processing. Many potential benefits could be derived from such discrimination. Quality classes would enable graduated pricing of shrimp. Excellent quality shrimp would be rewarded with a higher price while poorer quality shrimp would be penalized with a lower price (Ward, 1979). It could also improve the marketability of shrimp based on the potential shelf life and the physical condition of the shrimp . Overall it could provide the economic incentive within the industry to improve quailty.

Presently no single, quick and simple analytical method is considered acceptable to accurately evaluate quality before processing. Traditional methods such as aerobic plate counts and total volatile nitrogen require time and involve many steps. Therefore the industry relies on organoleptic evaluations such as smell and visual assessment to determine acceptability. While these subjective evaluations are useful they are subject to question. An objective determination to substantiate these evaluations would be desirable.

The purpose of this study was to look at several existing rapid and simple methods of evaluating shrimp quality and to develop some simple combination of these methods which could give rapid and accurate results. This report is a preliminary analysis of the study using 137 randomly selected subsamples from the 270 samples obtained. Further analysis of the data may provide a more acceptable combination and sequence of methods to be employed.

MATERIALS AND METHODS

The 137 samples of shrimp were collected from two packing plants

along the Texas coast, at four different times between August and December. Each sample was approximately five pounds and was removed from either iced boxes, iced in trucks or frozen bags. They were obtained before or during unloading into the thaw tank. Washes prior to this point were unknown. The physical condition of the shrimp was evaluated by subjectively sorting and weighing five pound samples into the following divisions: black spot, pigmented ("hot" shrimp), broken, pieces, soft shell, extraneous, other, and regular tails. Regular tails exhibit no physical defects and are considered the acceptable shrimp from the sample.

This procedure allowed for continuous sampling during processing and maximized the number of samples that could be observed for this analysis. After recording the weights of each division, three to six of the shrimp from the regular tail division of the sample were removed to a sterile Whirl-Pak bag for futher analysis, while the remainder of the five pound sample was returned for processing.

Shrimp removed from the sample for chemical analysis were refrigerated or frozen. The refrigerated samples were analyzed at the end of the workday, the next morning or frozen and analyzed later at Texas A&M University's Food Quality and Safety Laboratory at College Station. The freshness quality or chemical condition of the shrimp was evaluated by the following objective methods: pH, presence of sodium bisulfite (AOAC, 1980), direct microscopic count (Nickelson, 1975), ammonium ion concentration (Anonymous, 1978), salt and ammonium aroma. The pH, the only objective method data which has been evaluated at this time, was determined by the following procedure: Three shrimp were allowed to reach room temperature. Two readings were obtained on each of the three shrimp, one at the anterior end and another at the third segment. For the second reading, the shell was lifted away and the probe inserted into the top side of the shrimp. An Orion model 91-63 needle combination pH electrode and a Corning model 610A expandable portable pH meter or a Corning model 110 digital pH meter were used for measurements.

RESULTS AND DISCUSSION

Preliminary analysis of the random sample indicates that a possible simplistic procedure for quality assessment could be based on the percentage of regular tials and pH (these are the only two parameters whose data has been analyzed at this time). These two parameters were selected for evaluation based on observation of the shrimp over the harvesting season and on recommendation from experienced personnel in the shrimp industry. They represent both the physical and chemical conditons of the shrimp and are simple and rapid to perform. The percentage of regular tails indicates how much of the sample will pack out as tails with no physical defects,

representing the most desirable portion of the catch. From these shrimp, representing the best of physical conditon, the average pH determines the chemical condition of the sample. The relative physical and chemical conditions of the sample can then be compared to set standard categories. Figure 1 represents such categories.

The category levels 1, 2, and 3 boundaries for percentage of regular tails were set at 80% and 60%. Samples with greater than or equal to 80% regular tails were considered 1's, those between 60% and 80% were considered 2's, and those with less than 60% were considered 3's. These levels were set based on the percentage distribution observed in the samples over the season and with consideration of input from experienced personnel. Natural breaks in the distribution occur at approximately 80% and 60% forming a bell type distribution. Samples exhibiting 80% or better regular tails category pack out with a very high quality. Samples exhibiting less then 60% regular tails, category 3, pack out with low quality. The majority of the samples exhibited a category 2 acceptable quality, exhibiting between 80% and 60% regular tails.

Category levels 1, 2, and 3 boundaries for pH were set at 7.1 and 7.5, according to sample distribution, experienced personnel input, and with consideration of results from Bailey et al. (1956). The highest quality level, 1, had pH values less than or equal to 7.1; the average quality level, 2, had pH values between 7.1 and 7.5; and the lowest quality level, 3, had pH values greater than 7.5.

The overall quality category is obtained by using the percentage of regular tails and the pH value as coordinates in the graph of figure 1. Most samples fall into the shaded category areas 1, 2, and 3 with corresponding coordinataes 1:1, 2:2, and 3:3, where 1 equals the highest quality. When a sample falls outside of these areas then another parameter is used to help determine the sample quality. A sample falling above the shaded areas has a chemical quality less than the physical quality, therefore another chemical value, such as ammonium ion concentration, can be utilized. A sample falling below the shaded areas has a physical quality less than the chemical quality and analyzing the other physical divisions can determine the final pack out, therefore, indicating the appropriate quality category assignment.

Further analysis of the data may reveal more effective boundaries or parameters for this procedure. The effect and importance of each parameter will be accessed according to the industries needs and the procedure will be modified to reflect the most important of these relationships. Also the final procedure will have to be tailored, in such a manner that all the analysis determined necessary can be performed on a boats catch before or during processing.

This 1-2-3 quality category system should not be confused with the FDA's 1-2-3 class system based on indole levels as an indicator of decompostion, although they may fall in line with each other

(Anonymous, 1981).

The random sample category distribution, as it is determined by the above procedure, is illustrated in figure 2. Of the 137 samlples 32% were 1's, 64% were 2's, and 41% were 3's. At any time in a season this distribution may be skewed to one side or the other but overall a bell curve would be expected for the total season.

More analysis of the data and its relationships to the shrimp industry is needed. Data from the other procedures used (ammonia ion concentration, direct microscopic count, % black spot, etc.), has not been analyzed at the time of this report, but these parameters will be analyzed and their potentials reported at a later date. This investigation has and will strive to incorporate common objective tests with subjective tests already recognized in the industry to establish some fast, accurate and simple procedure to categorize the catches into clear-cut quality pricing groups. If higher prices are paid for good quality and poor quality penalized, the long term affect will be overall quality improvement of the product for all involved.

Preliminary analysis of the 137 randomly selected samples indicates a possible procedure that would utilize the pH of the sample and the percentage of regular tials in ranking the sample into categories of 1, 2, and 3 where 1 is the highest quality and 3 the lowest. Further analysis of the data is needed to support or modify this preliminary procedure. More indepth analysis of each parameter and its importance to the industries needs will be determined and incorperated, trying to produce an acceptable and favorable final procedure.

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Figure 1. Quality Guideline Category scheme based on Percentage of Regular Tails and pH.

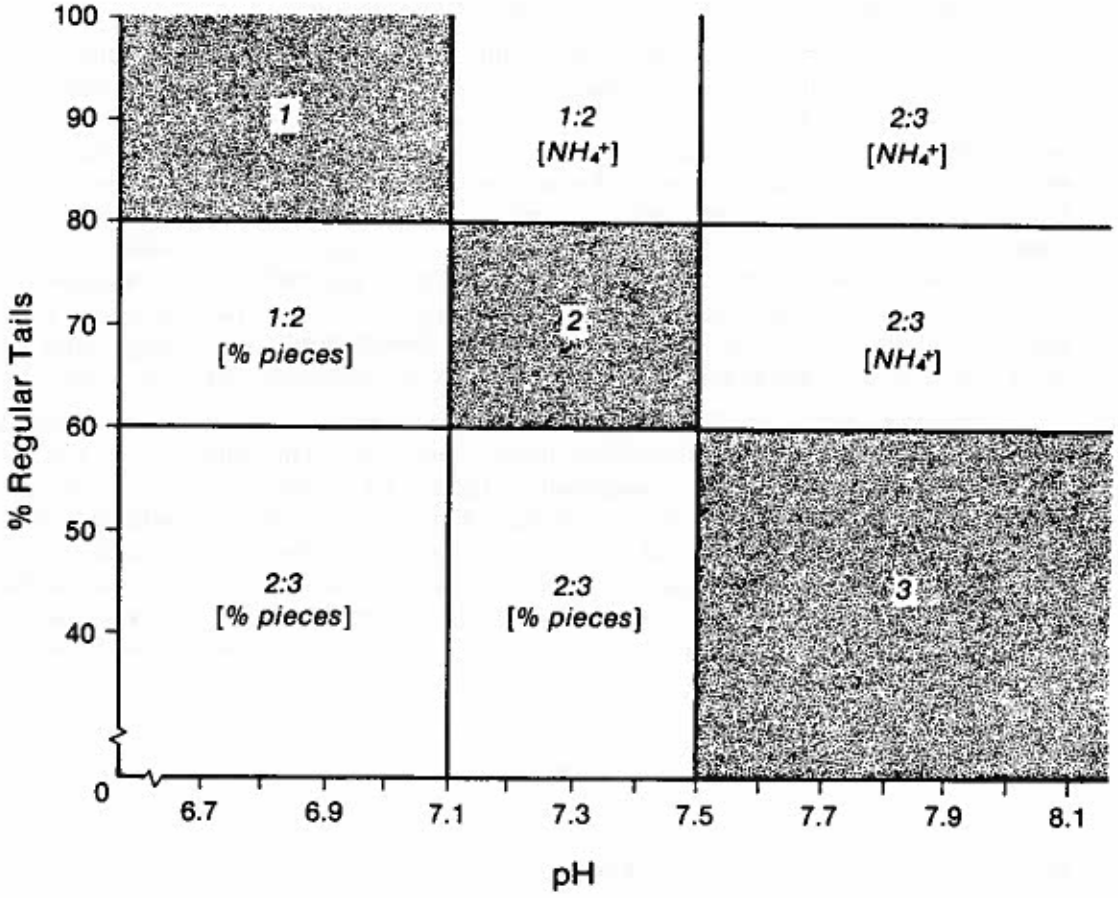
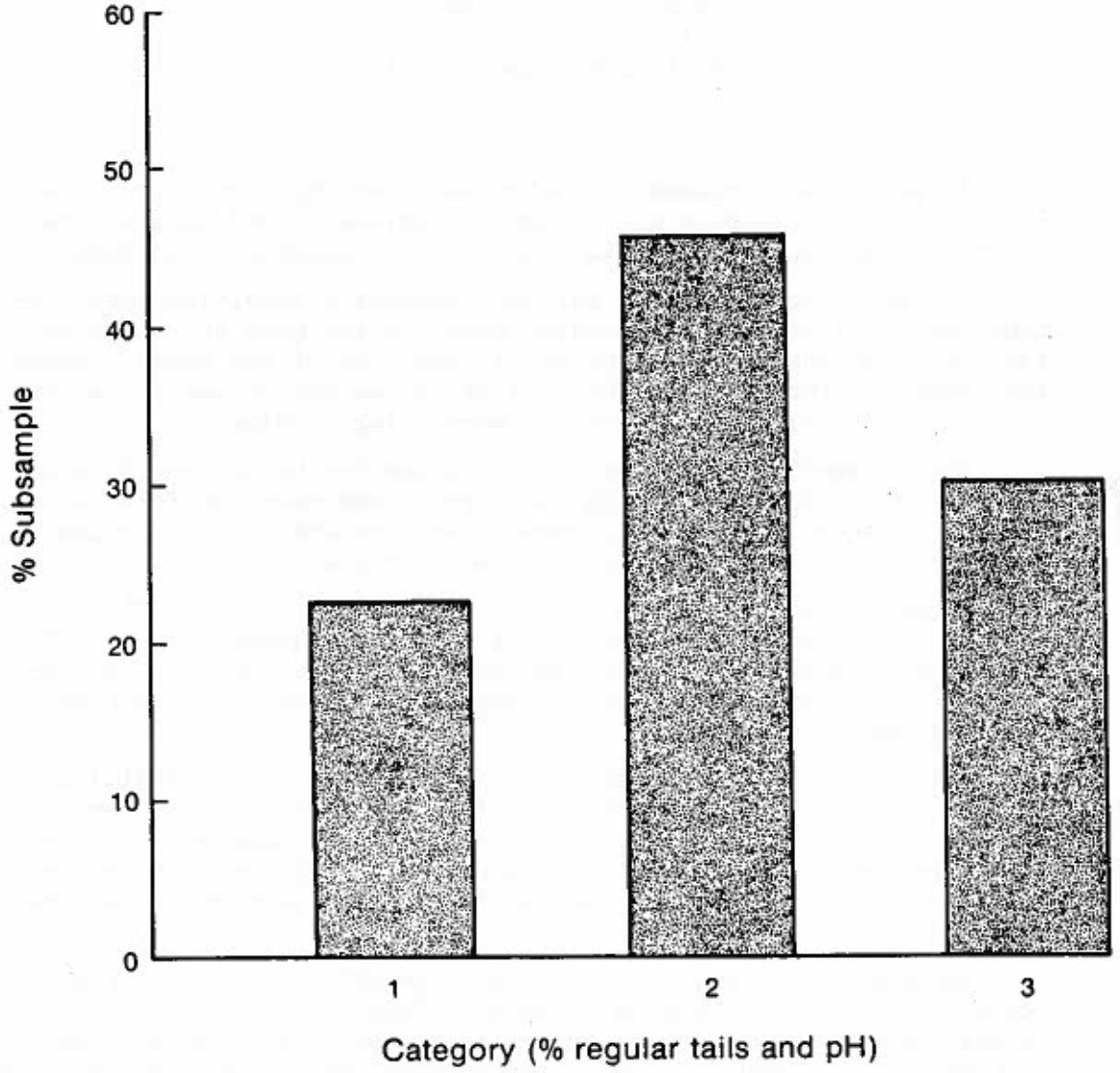


Figure 2. Percentage of Shrimp Quality Guideline subsample (137 random samples) for a category system using pH and Percentage of Regular Tails as parameters.



POND HARVESTING SYSTEM
SHRIMP FROM POND TO BOX IN MINUTES

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It should be stressed at the outset that the Sort-Rite "Aqua-Flo" Pond Harvest System was designed, engineered and manufactured to fill a distinct need in the aquacultural industry world wide.

The existing methods of harvest, generally used, involved fitting nets over the water discharge gates of the pond and catching the shrimp in the nets as they are flushed out of the ponds. Often this requires the gates to the pond be opened and closed at certain intervals, creating three distinct harvesting problems.

Exotic species of marine life that are now being used in aquaculture can often slip through the netting and move out into native waters, a hazard to the environment that more and more countries around the world are beginning to examine closely.

Oxygen depletion within the pond often occurs during the final stages of the net harvest causing the shrimp to either jump out of the ponds or burrow in. If burrowing occurs on a large scale, immediate reflooding of the pond is required, losing valuable time, manpower and pond yield.

The third problem of harvesting ponds under these conditions is the lack of a complete harvest. The slow pace of water removal and the intermittant opening and closing of the gates retards the shrimp movement. In general practice it is estimated that between 5% to 15% of the shrimp crop may be left in the pond and subsequently lost by the netting method.

In some countries, cast netting is practiced to reduce the pond population prior to the pond drainage. This creates problems within itself, in that the boats or trucks to transport the product have no ice nor even rudimentary facilities to keep the shrimp iced, and quality as well as yield values suffer. When shrimp are allowed to remain at a warm terperature after harvest, direct body weight loss will occur rapidly.

In building the pond harvest system, four goals were set forth: product quality; yield considerations; labor requirements; and mobility.

Quality was of primary importance in developing the pond harvesting system. The shrimp had to be harvested rapidly and on a continuous basis and iced as quickly as possible to retard cellular

breakdown and resulting body weight loss in the shrimp. This has been accomplished quite uniquely through a foot valve, pump and dewatering system in combination that deposits the shrimp directly from the pond and into a conveyorized ice bath bringing the temperature of the shrimp down to approximately 40°F immediately after removing them from the pond.

As a function of the quality factor, a section of this harvesting system is provided with an inspection area to remove unwanted marine species and trash from the shrimp. Crabs, of course, are an international problem in the shrimp ponds and aquaculture industry in general and if these crabs are allowed to remain in the harvest baskets with the shrimp, they can and will create havoc and the overall harvest quality and the economic recovery will be reduced.

Yield considerations were the second priority in the development of this system. It was noted earlier by use of the gate "net system", that 5% to 15% of the shrimp crop were left in the pond because the shrimp either burrowed in, jumped out of the pond onto the banks and died of natural causes, or fell prey to the ever present sea gulls.

With the "Aqua-Flo" Pond Harvest System 100% of the crop can be harvested from the pond maintaining a distinct economical advantage in the harvest. Also by immersing the shrimp quickly in an ice bath to lower the body temperature, the shrimp become immediately inactive and maintain an almost dormant state. This dormancy in effect retards the loss of body and tissue moisture of the shrimp and ultimately the product weight recovered from the harvest.

As an example of overall yield and recovery requirements; if shrimp are selling at $3.50 per pound, with an expected pond yield of 1,000 pounds per acre, a 5% yield loss through body moisture loss or failure to remove from the pond, equals $6,100.00 loss for a 40 acre pond per harvest.

Labor, necessary to harvest the aquacultural pond, was the third requirement considered. The "net catch" method is slow and cumbersome. It is also labor intensive and generally done in remote areas where security has become an increasing problem and sanitation is nonexistent. The "Aqua-Flo" Pond Harvesting System is capable of harvesting up to 3,600 pounds of shrimp per hour with five workers. (Two inspecting the product, removing the crabs, fish and natural pond debris; one worker weighing the shrimp; and two men boxing, icing/stacking the product, as well as keeping the conveyorized wash/ice tank properly iced).

Mobility of this unit was of course a prime consideration. Easily mobile with self-contained power supply through a diesel engine. As an option, the unit may be equipped with all weather protection and lighted for 24 hour a day operation, bringing this harvesting unit directly to the remote pond areas.

With the advent of polyculture, which has now been proven feasible, pond harvesting systems are even more important if not essential. When dual marine life species are harvested absolutely simultaneously, oxygen levels can be a critical factor, therefore, rapid harvest of the pond is essential and separation of the species at the harvest site is imperative to maintain quality and yield factors. Aquaculture on the scale we realize today is a new and expanding field and following rapidly is the natural evolvement of polyculture. Nations worldwide have spent millions of dollars in the development of the aquaculture industry from spawning and post-larval stages, feeding programs, pond management, genetic studies and disease and parasite control.

Sort-Rite is striving to complete the aquaculture cycle with updated and new harvesting techniques and systems that are needed today in the aquacultural world.

AN EVALUATION OF METHODS FOR THE RAPID DETERMINATION OF E. COLI IN THE EASTERN OYSTER (CRASSOSTREA VIRGINICA)

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INTRODUCTION

Being filter feeders, oysters accumulate microorganisms from their environment, thus consumption of oysters from waters polluted with human sewage has resulted in several well-documented outbreaks of various enteric diseases. For many years the sanitary quality of shellfish and shellfish growing waters was assessed by determining the density of coliform organisms (i.e. total coliforms). Realizing that the coliform group was quite heterogeneous and widely distributed in nature as well as in the intestinal tracts of warm-blooded animals, public health officials soon began to debate the merits of using an enumeration technique which would isolate a more restricted portion of the total coliform population, one which was more indicative of contamination from human sewage.

After four decades of debate, the elevated temperature test for fecal coliforms was adopted by the National Shellfish Sanitation Program and is now used routinely to assess the sanitary quality of shellfish and shellfish growing waters. However, this test still requires 72 hours to complete, and makes the assumption that most of the gas positive isolates are in fact E. coli, an organism closely associated with the feces of warm-blooded animals. It should be noted that E. coli itself is not normally pathogenic, but is used as an indicator organism because it 1) occurs in much greater numbers than pathogenic microorganisms in the intestinal tract, 2) has been shown to have similar survival properties in various fresh water and marine environments, and 3) is relatively easy to isolate in a short period of time.

More recent studies, however, have shown that in many shellfish samples there is a lack of correlation between organisms isolated by the elevated temperature method (i.e. fecal coliforms) and E. coli, the warm-blooded enteric microorganism. Thus, the isolates continue to be referred to as fecal coliforms, not E. coli.

This system worked fairly well until recently when several shipments of Gulf Coast oysters harvested in the summer and shipped as shell stock to the east coast for further processing were either detained or destroyed because of excessively high fecal coliform counts (i.e. in excess of 230 MPN fecal coliforms/100 grams meat). The question arose as to the sanitary

significance of these findings since the oysters came from waters which met bacteriological water quality standards for shellfish harvesting.

At about the same time several researchers were finding that post harvest oysters, particularly those that were temperature abused on the boat or in shipment, supported the outgrowth of fecal coliform organisms. However, these were not further confirmed as E. coli.

This brings us up to the most recent Interstate Shellfish Sanitation Conference held last summer, which concluded that, for an interim one year period, the fecal coliform standard (i.e. 230 MPN fecal coliforms/100 gms oyster meat) would be used as a screening procedure, but not as a basis for regulatory action; and further, that regulatory action would be taken only if these organisms proved to be E. coli upon further examination. Obviously then, a more rapid test for E. coli would benefit the industry, since shellstock would not be held up because of lengthly bacteriological analyses.

For this reason, we have been evaluating rapid procedures for the enumeration of E. coli. In this study we compared two rapid methods for the enumeration of E. coli with the APHA 10 day standard methods (IMViC) procedure.

MATERIALS AND METHODS

Sample Preparation

Approximately 200 oysters (C. viginica) were harvested from Chocolate Bay, a part of the Lavaca Bay System, which is classified as polluted and therefore closed to oystering (Figure 1). The oysters were transported to the Seafood Technology Laboratory in Corpus Christi where they were stored overnight at room temperature, then subjected to bacteriological analyses the following day.

Oysters were scrubbed and alcohol sterilized before shucking. Twelve shucked, drained shellfish meats were blended with an equal weight of 0.5% sterile peptone diluent for 90 seconds. Twenty grams of this homogenate was then added to 80 ml of peptone water. Two additional dilutions were prepared from the original 1:10 dilution by adding 10 ml aliquots to 90 ml of peptone water.

Samples were subsequently examined for fecal coliforms by a modified A-1 method (ISSC Ad-Hoc Committee on Processing and Distribution) and the standard method (APHA Recommended Procedure for the Examination of Seawater and Shellfish). All positive fecal tubes were further tested for E. coli according to the respective procedures for each method. In addition, the same samples were examined for E. coli using the direct count method of Anderson and Baird-Parker (1975).

ISSAC A-1 Procedures

A five tube MPN test at four dilutions (double strength A-1 medium for 10 ml inoculations) was used. Samples were incubated at 35°C in an air incubator for 3 hours, then placed in a 44.5 ± 2°C water bath for

24 hours. Following incubation, all positive tubes were streaked onto Levine's EMB agar plates for isolation. Inverted plates were incubated at 35°C for 20 hours. One typical E. coli colony was picked from each plate and inoculated onto a Standard Methods Agar (SMA) slant, tryptone broth and Simon's citrate slant. Following 24 hours incubation at 35°C the tubes were examined for indole and growth on citrate. Tubes showing a positive reaction for indole and negative for citrate were counted as E. coli. This procedure is illustrated in Figure 2.

APHA Procedure

A five tube MPN test at four dilutions (double strength Lauryl Sulfate Tryptose (LST) for 10 ml inoculations) was used. Inoculated tubes were incubated at 35°C and checked for gas production after 24 and 48 hours. All gas positive tubes were transferred to E. C. medium and incubated in a 44 ± 2°C water bath. Samples were checked for gas production following 24 and 48 hours incubation. All gas producing tubes were streaked onto Levine's EMB agar plates for isolation. Following 24 hours incubation at 35°C two typical colonies were picked from each plate and transferred to SMA slants and LST broth. These were incubated for 24/48 hours at 35°C. Cultures from all slants with corresponding gas positive LST tubes were inoculated into tryptone broth, MR-VP medium and Koser's citrate for IMViC reactions. All cultures showing reactions typical for E. coli (++-- or -+--) were used in determining MPN per 100g. (No type II isolates were found). The procedure is illustrated in Figure 3.

Anderson and Baird-Parker Procedure

Tryptone Bile Agar (TBA) was prepared and poured into petri dishes as described by Anderson and Baird-Parker (1975). Cellulose acetate filter membranes (pore size 450nm; 90mm diameter) were cut to fit the agar surface and smoothed onto the TBA media with a sterilized spreader. 7 ml of a 1:1 dilution (original homogenate) was pipetted onto the membranes in 7 petri dishes (1 ml per plate) and counted as one dilution (i.e. 3½ grams meat). The homogenate was spread completely over the membrane with a sterile spreader. Inoculated plates were placed in sterile whirl paks and incubated in a water bath at 44° ± 1°C for 18 hours. Following incubation 2 ml of 5% p-dimethylaminobenzaldehyde in 10% aqueous HCl was pipetted into the labeled lids. Indole positive colonies stained pink within five minutes. Colonies yielding a positive reaction were counted and the number of E. coli per gram determined. This number was multiplied by 100 in order to equate it to the other two methods employed in E. coli determinations. This procedure is outlined in Figure 4.

Recovery of E. coli from Pure Culture

The reliability of recovery on Anderson and Baird-Parker medium was determined through the use of a pure culture of E. coli. The culture was prepared using an organism isolated through the standard IMViC reactions and maintained on Standard Methods agar slants. The slant culture was inoculated into 1500 ml of Trypticase Soy Broth and allowed to grow at 35°C for 5½ hours. Following incubation, serial dilutions

were prepared in 0.5% peptone diluent. Dilutions of 10^4 - 10^7 were plated on both Standard Methods (SM) agar plates and on Tryptone Bile Agar (TBA) according to the Anderson and Baird-Parker method. 1 ml of the appropriate dilutions was pipetted onto the TBA plates and 0.1 ml was pipetted onto the SM plates. TBA plates were incubated as previously described and SM plates were incubated at 35°C for 24 hours. Following the incubation period, colonies on the SM plates were counted and multiplied by the dilution factor to determine number of E. coli per ml. Membranes from the TBA plates were placed onto the reagent in the lids of the petri dishes as previously described. Following staining, colonies were counted and multiplied by the dilution factor to obtain number of E. coli per ml of culture.

RESULTS AND DISCUSSION

Table 1 shows results of the tests comparing the three methods for the recovery of E. coli from oyster meats, as well as results of confirmation of gas positive fecal tubes as E. coli. Identical unconfirmed and confirmed counts were obtained in 70% of the A-1 samples and 80% of the Standard Methods (IMViC) samples. Table 2 shows statistical data comparing the two MPN tube methods, and indicates no significant difference between these methods for the recovery of unconfirmed E. coli from positive fecal tubes. Table 3, however, shows that the membrane overlay plate method recovered significantly fewer E. coli organisms than did either MPN tube method. Data in table 4 indicate that the recovery of E. coli isolates grown in enriched medium and enumerated on the Anderson and Baird-Parker plates did not differ significantly from Standard Plate Count numbers.

Prior research (Yoovidhya and Fleet, 1981) confirmed that 95% of the pink staining colonies isolated on the Anderson and Baird-Parker medium were in fact E. coli. Using a method of imprinting the filters on Red MacConkey Agar plates before indole staining, they found no significant difference between unconfirmed and confirmed E. coli when testing over 30 samples of Sydney Rock oysters, Crassostrea commercialis.

These findings differed from the results of our study, but the authors point out that they were working with freshly harvested oysters and suggested a resuscitation period may be necessary for stored or frozen samples. As pointed out in the methods section, we used oysters which had been stored overnight at room temperature, and we did not include a resuscitation step in our membrane filter procedure. In both of our tube tests, A-1 and Standard Methods, the samples were initially exposed to a lower temperature for a period of time before incubation at 44.5°C. Also, Anderson and Baird-Parker medium, which contains bile salts, could have been more selective than either the A-1 medium or the LST broth used for initial isolation in the Standard Methods procedure.

Other studies (Andrews et. al., 1975, Haven, et. al., 1978), however have shown higher recovery of E. coli from C. virginica using A-1 vs. Standard Methods procedures.

In a study using oysters harvested in Alabama in the summer of 1977 Saba et. al. (1977) found no significant difference between Standard Method and A-1 positive tubes when isolating unconfirmed E. coli (i.e. fecal coliforms) from oysters. However, they subsequently identified only about 50% of the gas producing isolates as E. coli, most of the remainder typing out --++.

These data from oysters are similar to those from a sanitary survey of the waters in Mobile Bay where Andrews and Presnell (1971) found good agreement between the A-1 and Standard Methods procedures for isolation of fecal coliforms, but recovered only 39% E. coli from gas positive tubes.

However, in cooler waters that are not rich in carbohydrates from industrial or domestic pollution most sanitary surveys have generally confirmed from 75 to 90% fecals as E. coli (Menon et. al., 1975). Adams et. al. (1977) verified approximately 90% of fecal coliforms isolated from east coast estuaries as E. coli.

More tests used to differentiate E. coli from other thermotolerant coliforms rely on the ability of E. coli to produce indole from tryptophan at an elevated temperature. However, as Dufour and Cabelli (1975) pointed out in a paper describing a rapid membrane filter technique for differentiating various thermotolerant coliforms, about 16% of their Klebsiella isolates were indole positive, and they suggested a preliminary non-destructive urease test to distinguish between these organisms, followed by confirmation of E. coli through indole production. In the modified A-1 method we used, we further differentiated between fecal coliforms and E. coli, type 1, by testing gas positive cultures on citrate slants.

The purpose of this brief review is to point out some of the conflicting data and to pose the question which was the topic of so much discussion at the First Interstate Shellfish Sanitation Conference, namely "what is the sanitary significance of non-E. coli fecal coliforms?"

Presnell and Brown (1977) in their study of shellfish growing waters of Mobile Bay concluded that Klebsiella pneumoniae and non-E. coli coliforms were an integral part of the fecal coliform group and were significant indicators of the presence of fecal waste. Likewise, Menon et. al.(1975) pointed out that ten percent of the coliform flora in feces is non-E. coli and that K. pneumoniae is found in 30-40% of human and other warm-blooded animal intestinal tracts. They concluded that the fecal coliform test might therefore be preferable to that for E. coli in assessing the sanitary quality of shellfish and shellfish growing waters.

Summarzing these findings, one might conclude that the industry and public health organizations have been prudent in retaining the fecal coliform test for the evaluation of shellfish growing waters. However, what about the embargoed oysters and the interim E. coli standard for regulatory action on oyster meats referred to earlier?

This action is also probably justified since a recent FDA study (1983) showed that there was a dramatic increase in the fecal population in oysters while stored on the deck of the boat during the summer. E. coli populations on the other hand were largely indeterminate (i.e. less than 18 per 100 gms), and did not correlate with fecal levels from harvest, through boat storage, or subsequent shipment. The questions to be answered here are: if the fecal coliforms growing out in stored oysters are not E. coli, then what are they and what is their sanitary significance?

Our results from this one test showed that the recovery of E. coli from oysters using the modified A-1 method compared favorably with the lengthy IMViC procedure, and that there was no significant difference between unconfirmed and confirmed E. coli using either tube method. Recovery of E. coli (type 1) employing the Anderson and Baird-Parker membrane plate method, however, was significantly lower than either tube method. Obviously, more samples from various locations, harvested throughout the year, will have to be evaluated before any conclusions can be reached regarding the comparability of these methods for the recovery of E. coli in oysters.

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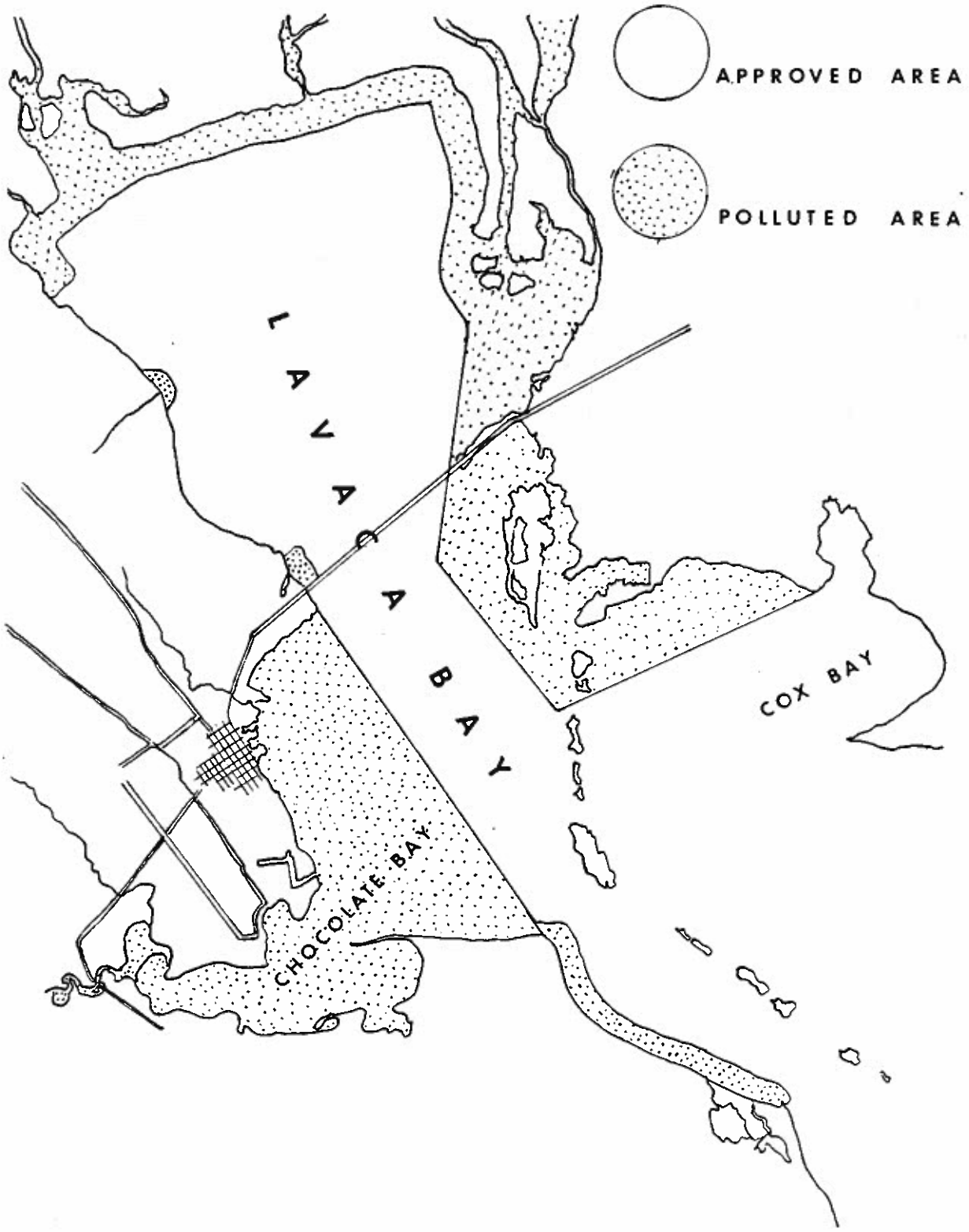
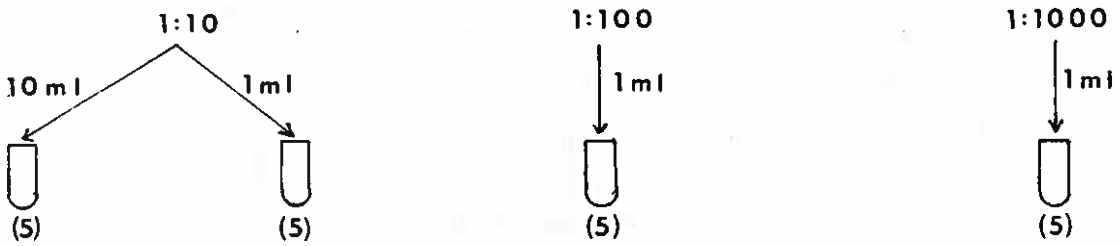

APPROVED AREA
POLLUTED AREA
LAVALA BAY
COX BAY
CHOCOLATE BAY

A-1



Incubate
3hrs 35°c
24hrs 44.5°c

Gas +

Levine's EMB
18-24hrs 35°c

Pick One Typical Colony

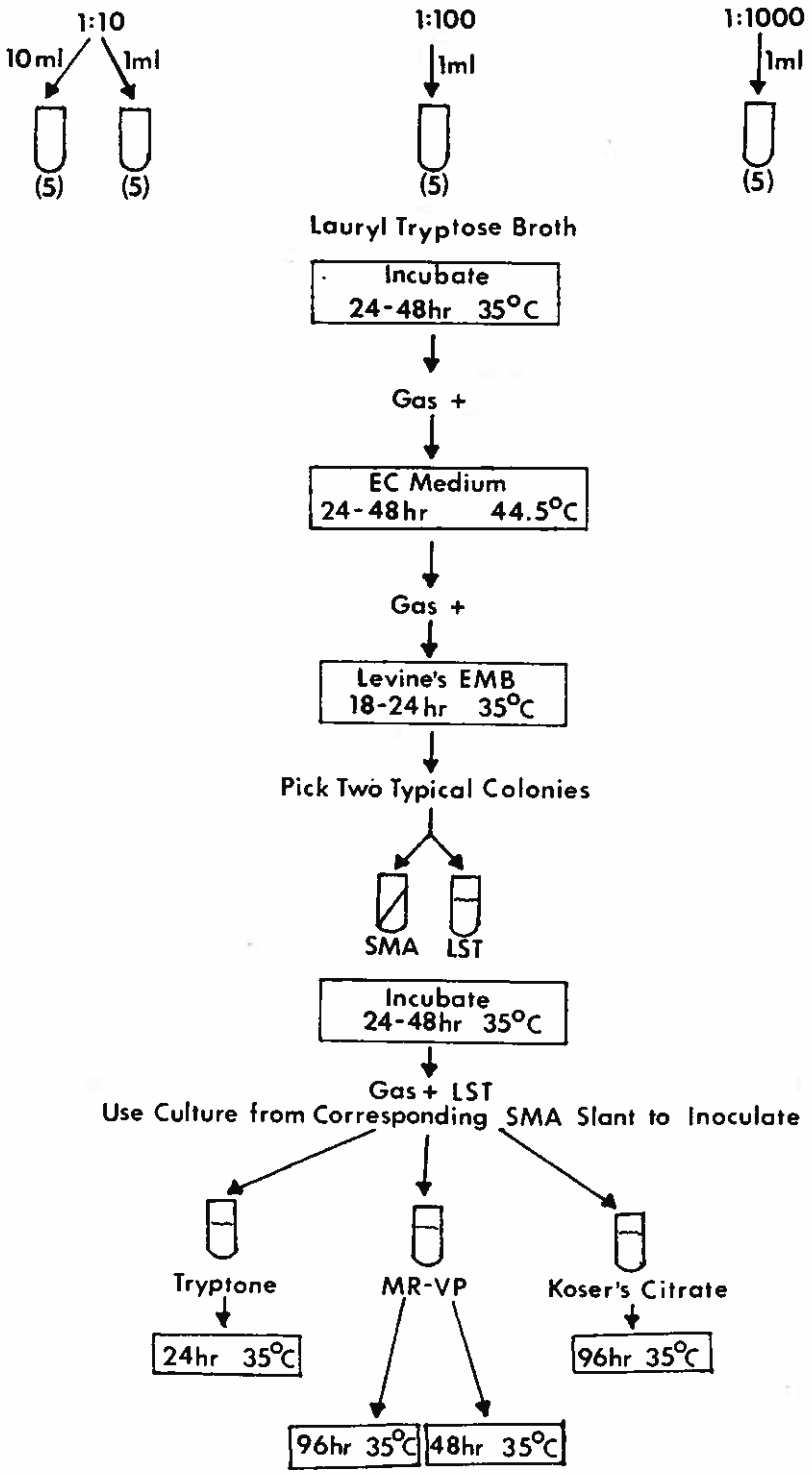
Simmons Citrate

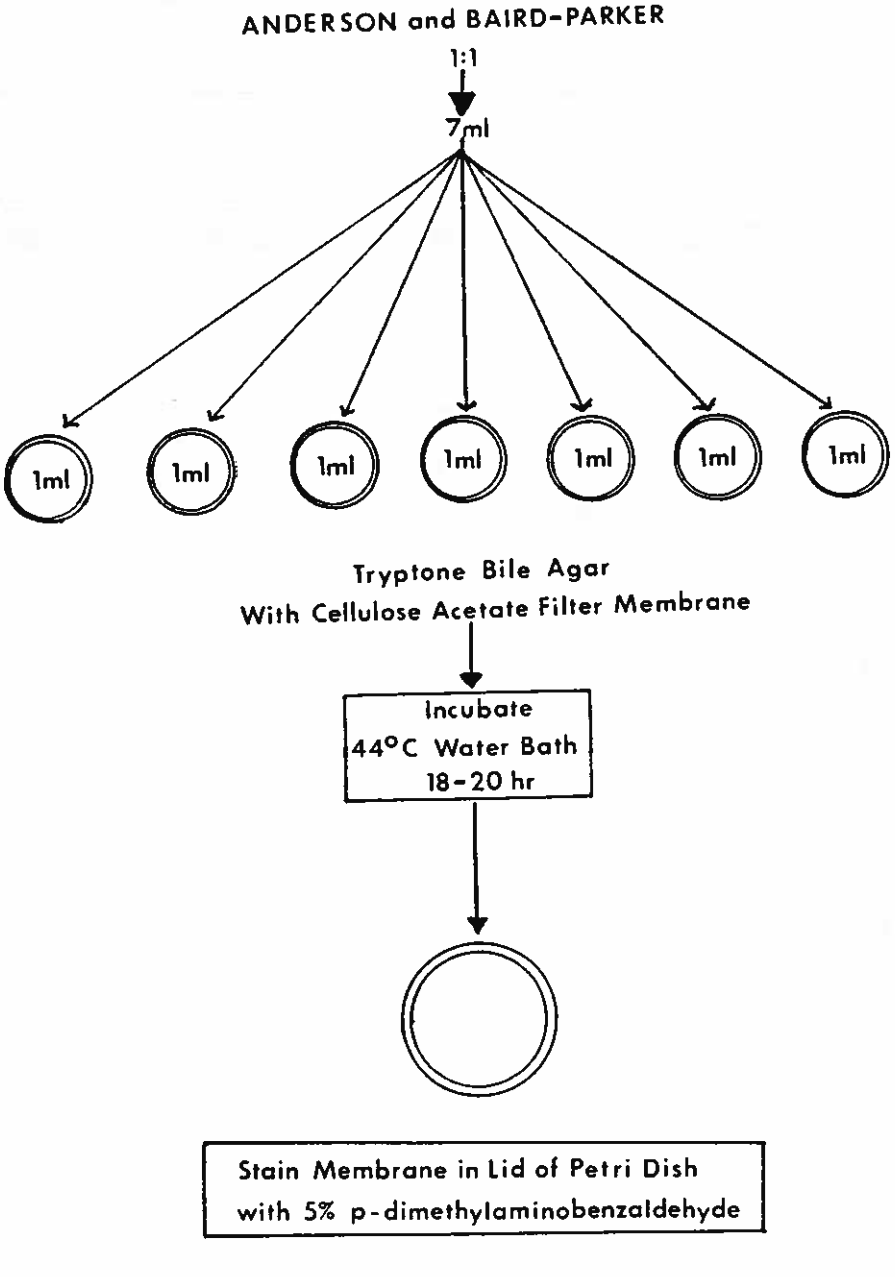
Tryptone

Standard Methods Agar

Incubate
24hrs 35°c

STANDARD METHOD




ANDERSON and BAIRD-PARKER
1:1
7ml
1ml
1ml
1ml
1ml
1ml
1ml
1ml
Tryptone Bile Agar
With Cellulose Acetate Filter Membrane
Incubate
44°C Water Bath
18-20 hr
Stain Membrane in Lid of Petri Dish
with 5% p-dimethylaminobenzaldehyde

Fecal coliforms and E. coli isolated from oysters using three methods of enumeration.

	A - 1		IMViC		Anderson & Baird Parker
	Fecal Coliform	E. coli	Fecal Coliform	E. coli	E. coli
Sample*	MPM/100g	MPM/100g	MPM/100g	MPM/100g	Organisms/100g
A	790	790	780	780	200
B	1700	1700	330	220	86
C	1100	330	1600	1600	257
D	790	790	490	490	200
E	1100	1100	1300	1300	257
F	490	490	1300	1300	286
G	490	490	490	490	314
H	490	330	1300	790	200
I	490	490	330	330	200
J	1300	490	330	330	343

* Each sample contained 12 oysters (meat only).

t - test for the recovery of E. coli from a pure culture using the direct plate method of Anderson and Baird-Parker.

Sample 1

	Aerobic Plate Count	Anderson & Baird-Parker
	6.7×10^8	6.0×10^8
	8.9×10^8	5.0×10^8
Mean	7.8×10^8	5.5×10^8

No significant difference between means

$.05 < p < .10$

Sample 2

	Aerobic Plate Count	Anderson & Baird-Parker
	5.8×10^8	4.6×10^8
	5.7×10^8	5.8×10^8
Mean	5.8×10^8	5.2×10^8

No significant difference between means

$p > .50$

t test performed on transformed data.

Analysis of variance and Duncan's multiple range test for fecal coliforms and *E. coli* determined by the modified A-1 and IMViC tests.

Source	SS	d.f.	MS	F
Between	0.076	3	0.025	0.393
Within	2.322	36	0.064	

Treatment	Mean
A-1 *E. coli*	700.00 a
A-1 fecal coliforms	874.00 a
IMViC *E. coli*	763.00 a
IMViC fecal coliforms	825.00 a

Means with the same letter are not significantly different.

Alpha Level = 0.05

p = .7586

ANOVA performed on transformed data.

Analysis of variance and Duncan's multiple range test for E. coli determined by three methods of enumeration.

Source	SS	d.f.	MS	F
Between	1.336	2	0.668	12.083
Within	1.493	27	0.055	

Treatment	Mean
A-1	700.00 a
IMViC	760.00 a
Anderson & Baird-Parker	234.300 b

Means with the same letter are not significantly different.

Alpha Level = 0.05

p = 0.0002

ANOVA performed on transformed data.

PRELIMINARY STUDY ON THE ISOLATION AND ACTIVATION OF POLYPHENOLOXIDASE FROM DEEP SEA CRAB (Geryon sp.)

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INTRODUCTION

Development of a deep sea crab fishery in the Gulf of Mexico must consider preventing black discoloration on the flesh from occurring during harvesting, handling and/or storage. Black discoloration develops on iced deep sea crabs after 24 hr and can be marginally inhibited using sodium metabisulfite dipping (2). The black discoloration commonly referred to as "melanosis or black spot" is caused by the enzyme polyphenoloxidase (PO). PO catalyzes the oxidation of phenolic compounds which polymerize to form the pigment melanin (3,4). In crustaceans, these enzymes play a physiological role in sclerotization of the shell after molting (7) and certain stages of the molting cycle, especially in shrimp, affect the degree of susceptibility to melanosis (3).

Although, melanosis produces no off-flavors, the discoloration is enough to cause consumers to reject the product. This work was undertaken to characterize the polyphenoloxidase system from deep sea crab (Geryon sp.).

MATERIALS AND METHODS

Deep sea crabs (Geryon sp.) were caught in the Gulf of Mexico. They were cleaned, ice transported to Gainesville, Florida and butchered into half sections. The half sections, consisting of a body with four legs and one claw were stored in plastic bags at -30°C until they were extracted. Before extraction the crab sections were thawed on ice for 24 hr. Crab sections were covered with cheese cloth, placed in a hydraulic press and squeezed under 15-20 tons force. The liquid extracted (5-25 ml) was collected at 4°C and centrifuged at 19,000 x g for 20 min at 4°C. The supernatant was collected and assayed for polyphenoloxidase activity according to Savagaon and Sreenivasan (6) with some modifications. The assay system consisted of 5 mM DL-3,4-dihydroxyphenylalanine (DL-dopa) in 0.05 M potassium phosphate buffer, pH 7.0 with and/or without 10% Coralase (Rohm-Tech).

Polyphenoloxidase was assayed using a Perkin-Elmer lambda 3 uv/vis spectrophotometer with a model 561 Perkin-Elmer strip chart recorder. Enzyme activity is expressed as the change in absorbance at 475 nm in 1 min at 22-24°C.

RESULTS AND DISCUSSION

Protease (Coralase) was capable of activating the polyphenoloxidase (PO) activity from deep sea crab (Fig. 1). The crude extract demonstrated very little PO activity, while crude extract with coralase showed a 25-fold increase in PO activity (Fig. 1). Initial velocities (v_o) for crude extract with and without coralase were 0.125 and 0.005 $\Delta A_{475\ nm}$ /min, respectively. A control consisting of coralase and assay mixture demonstrated no change in absorbance over the 5 min reaction period. Savagaon and Sreenivasan (6) showed a very similar activation process for PO isolated from lobster and shrimp shells and cephalothorax.

Crude extract (0.1 ml) was mixed with 2.8 ml DL-dopa and 0.1 ml protease (Coralase) at various concentrations (0-10%). PO activity from shrimp and lobster was shown to be sigmoidal with respect to varying concentrations of trypsin (6). However, data in figure 2 show that PO activity from crab increased linearly with varying coralase concentrations, again, demonstrating the dependency of PO for protease activation.

Enzyme activity also increased linearly with respect to relative protein concentration (Fig. 3). As the extract concentration increased from 0-50%, the v_o increased linearly for PO. Boiling the crude extract for 10 min resulted in a 100% loss in enzyme activity. A preliminary indication of an enzyme-mediated reaction is the ability of PO activity to increase linearly with varying protein concentrations and be reduced upon boiling.

A thermostability test was performed on the PO from crab. To two test tubes containing crab extract; protease (0.1 ml) was added to one (tube 1), while water (0.1 ml) was added to the other (tube 2). The tubes were placed in a constant temperature water bath for 30 min at the following temperatures (30, 40, 50, 60 and 70°C). After incubating, the tubes were placed in an ice bath (4°C) for 5 min and then assayed for PO activity. Samples from tube 1 and 2 were assayed using 5 mM DL-dopa containing protease, however, samples from tube 1 were also assayed using 5 mM DL-dopa without protease. Heating the extract (tube 2) between 30

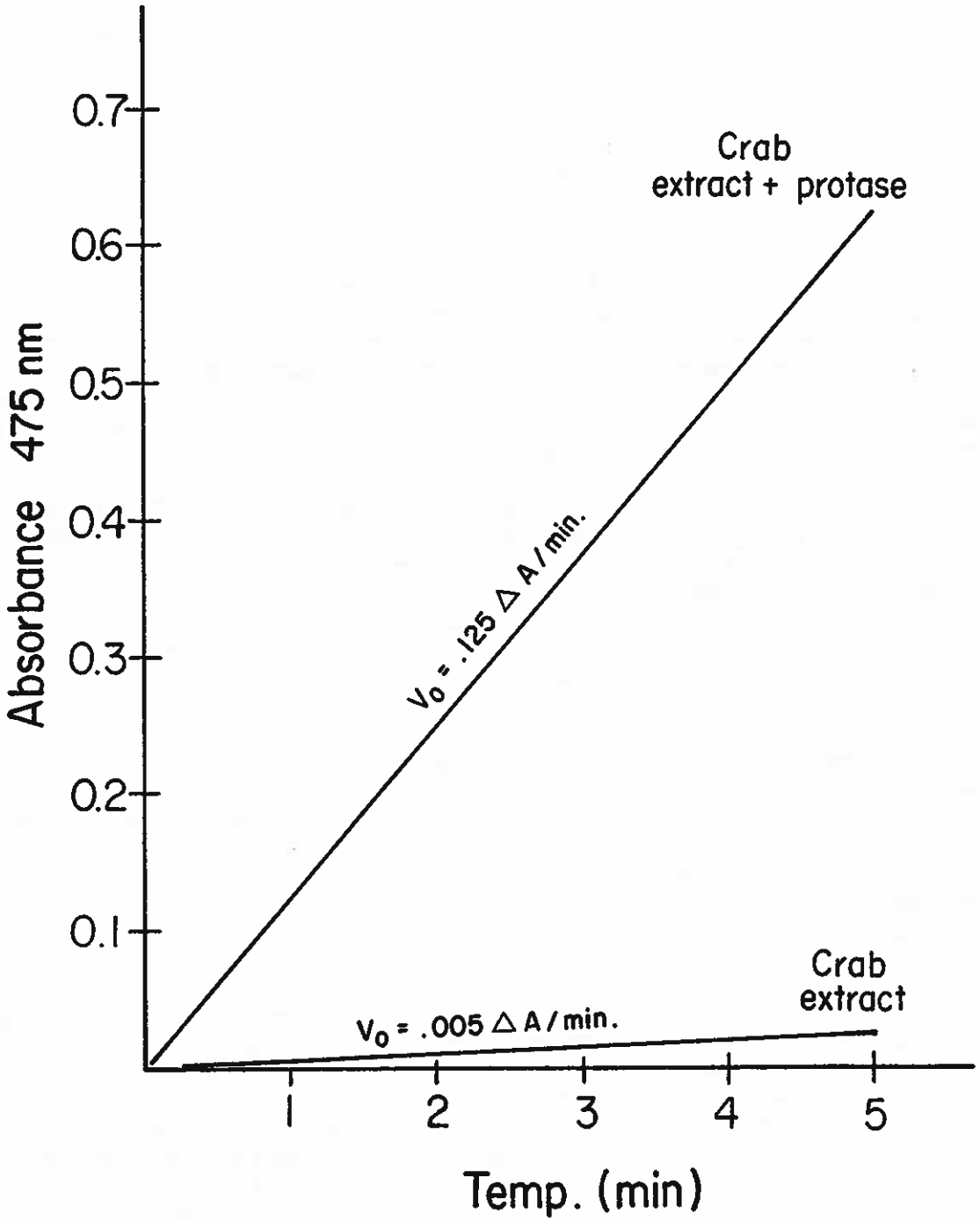

Figure 1. Activation of polyphenoloxidase by an in vitro protease (Coralase). Each curve was taken from individual recordings and is representative of over 10 additional trials.

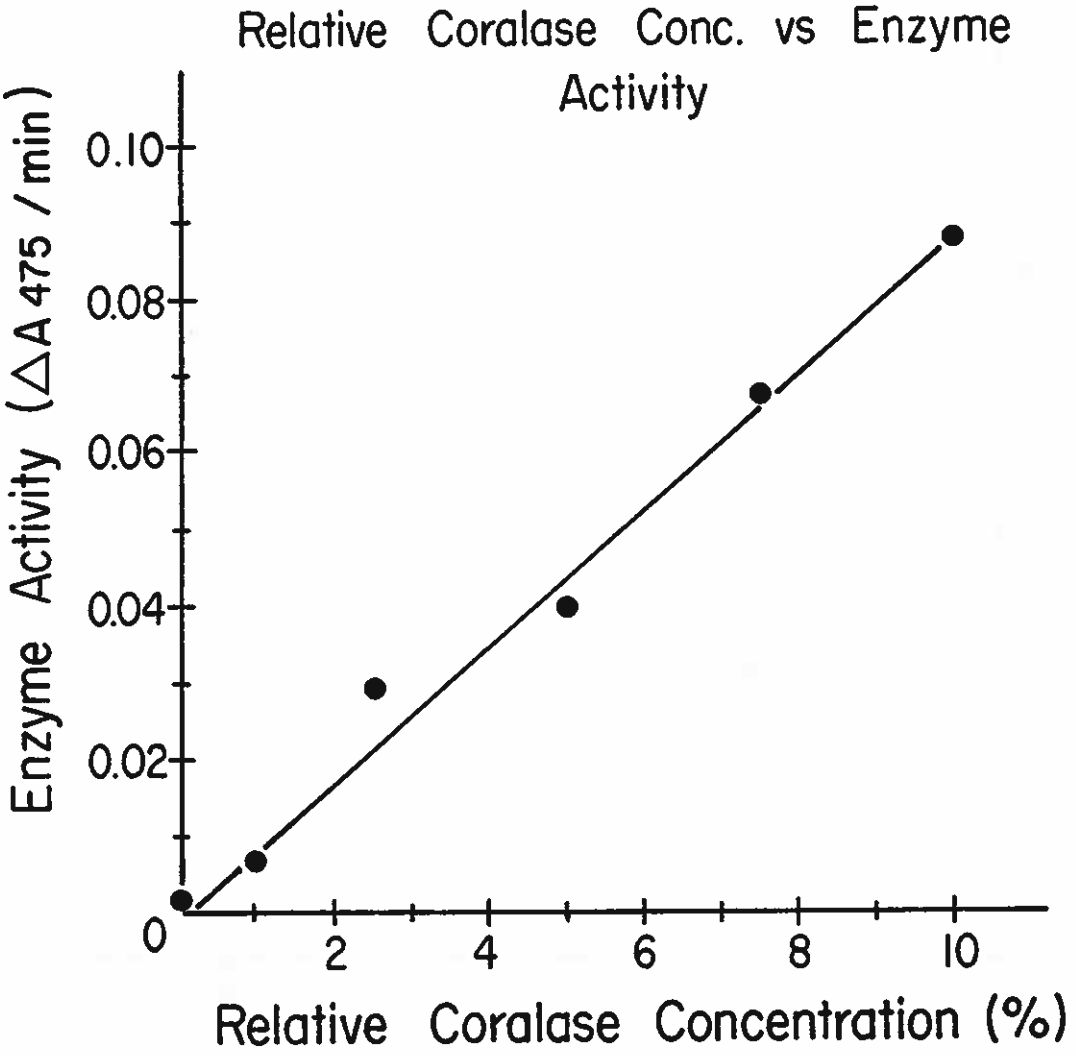

Figure 2. Polyphenoloxidase activity in the presence of relative coralase concentrations (0-10%).

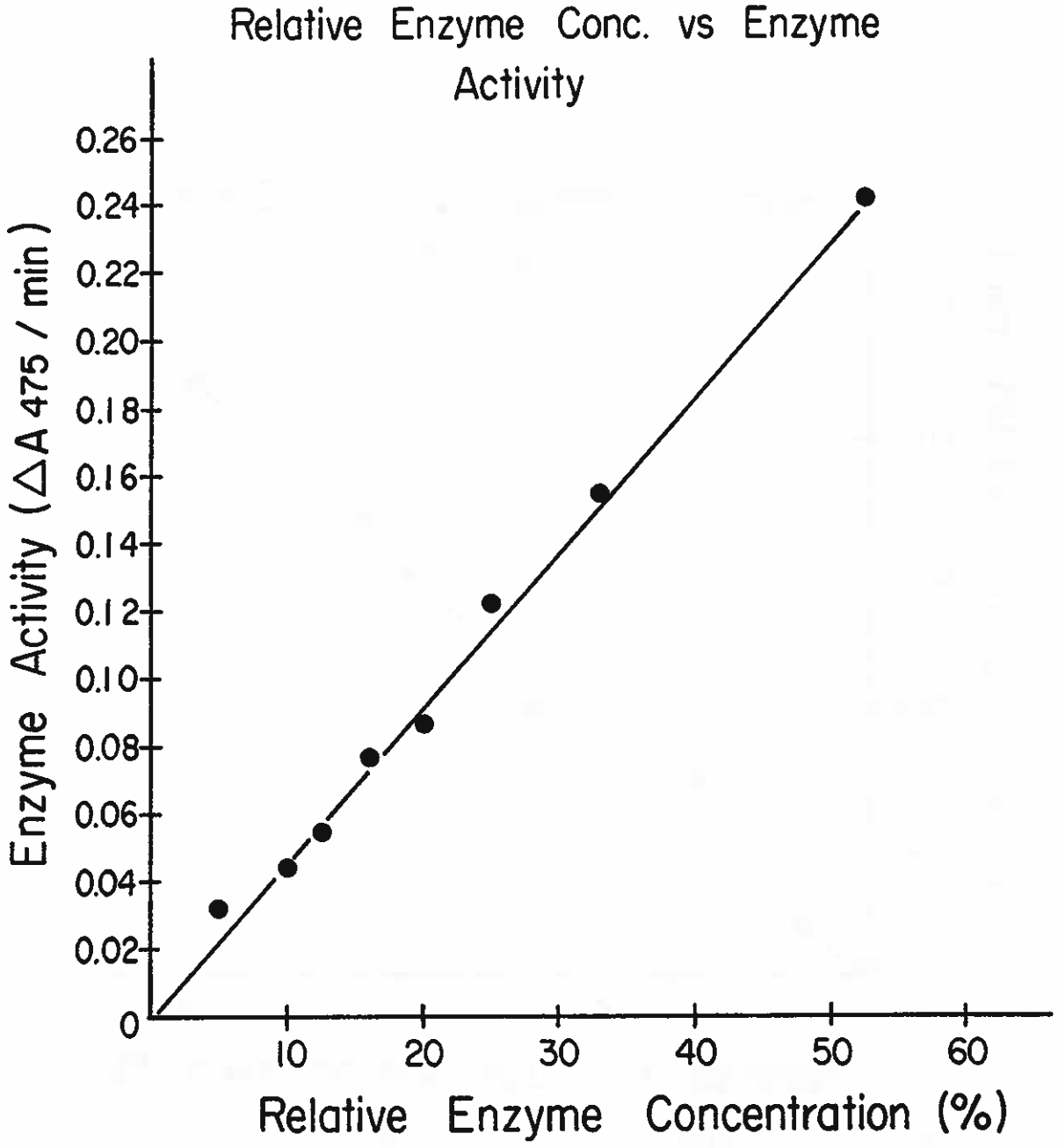

Figure 3. Polyphenoloxidase activity versus relative extract concentrations (0-50%).

and 50°C resulted in an increase in PO activity (Fig. 4). Maximum activity occurred at 40°C, with vo going from 0.200 at 30°C to 0.300 $\Delta A_{475\ nm}$/min at 40°C. This type of phenomena was demonstrated by Bailey et al. (1) in shrimp extracts. They attributed this to the proenzymatic nature of PO and active centers being exposed by the heat process. Total inactivation of PO from shrimp head extracts occurred at 50°C (5), however, deep sea crab PO appeared to be slightly more stable to heat treatment. Total inactivation occurred at 70°C (Fig. 4).

The thermostability curve for PO in the presence of protease demonstrated that protease is essential for activity. Figure 4 shows that PO activity declines at a greater rate when protease is present during incubation. However, if protease is also added to the assay mixture then very little change from the normal thermostability curve for PO resulted. Also, the increase in PO activity at 30°C for the extracts with protease versus the extract without protease demonstrates that activation of PO is occurring (Fig. 4).

CONCLUSION

PO from deep sea crab appears to follow the simple analyses for enzyme-mediated reactions. The enzyme apparently requires at this time an in vitro protease for activation to occur. If endogenous proteases do exist in the tissue then it may be possible to effectively control these enzymes, thereby, preventing melanosis from resulting.

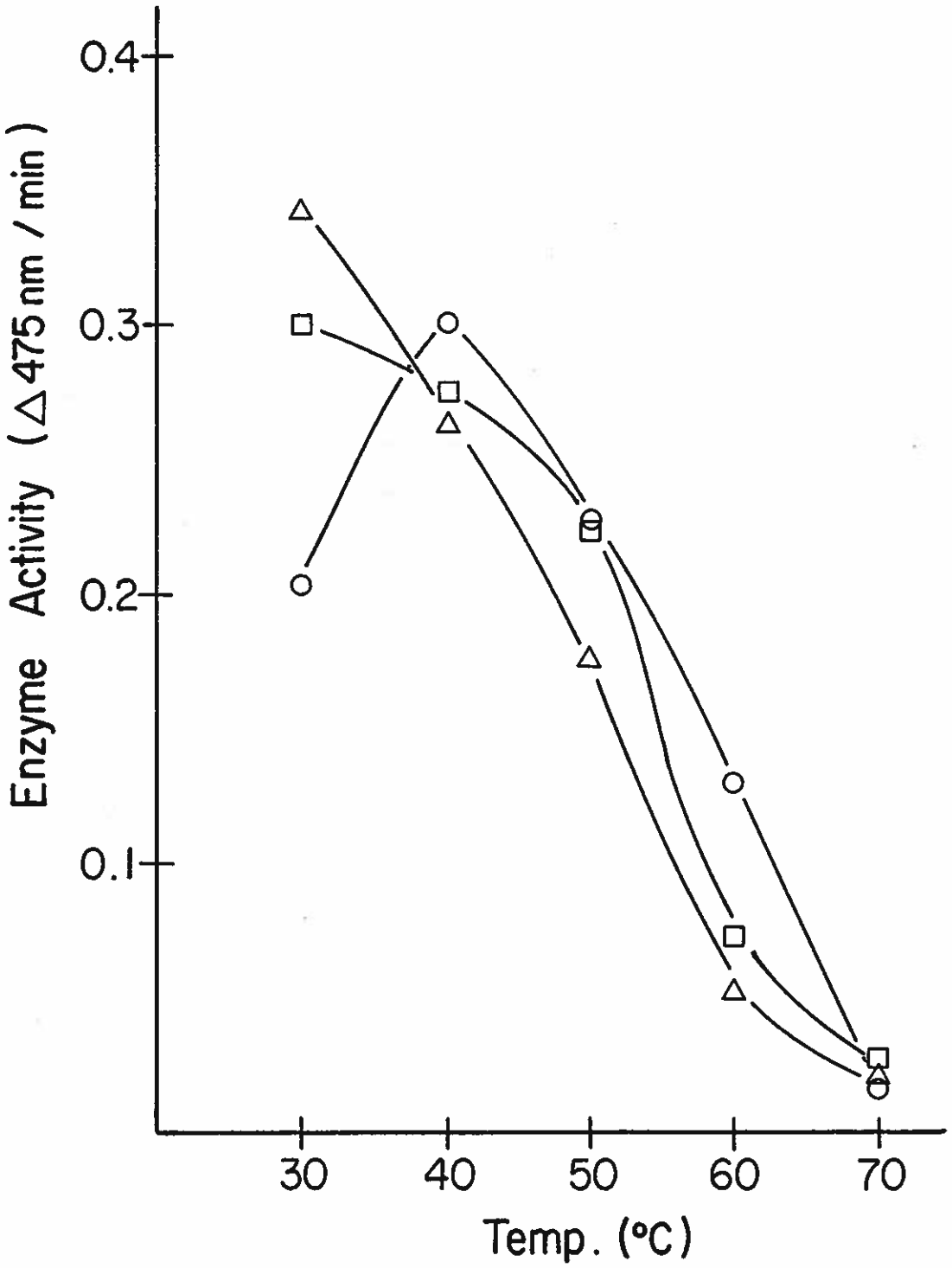

Figure 4. Thermostability curve for polyphenoloxidase at various temperatures. Crab extract (0.1 ml)+assay mixture (2.8 ml)+ protease (0.1 ml) (O-----O); Crab extract with protease (0.1 ml)+ assay mixture (2.8 ml)+protease (0.1 ml) (□-----□); Crab extract with protease (0.1 ml)+assay mixture (2.8 ml)+H_2O (0.1 ml) (Δ---Δ).

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NUTRITIONAL, CHEMICAL, MICROBIOLOGICAL, AND ORGANOLEPTIC CHANGES IN BREADED SHRIMP STORED IN WHOLESALE AND RETAIL FREEZERS

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INTRODUCTION

The quality and nutrition of frozen breaded shrimp available to the consuming public has been a recent concern of breaded shrimp manufacturing companies, state and federal regulatory agencies, retail merchants, and consumers themselves. Breaded shrimp manufacturers were convinced that they were producing a safe, nutritious, and appetizing product for public consumption. Periodic consumer complaints and regulatory actions led the processors to believe that the consuming public was not necessarily purchasing the same quality product that left their manufacturing facilities. Two previous papers, Rao et al., 1975 (10) and Williams et al., 1981 (14) addressed the problems encountered with moisture migration within breaded shrimp which caused the shrimp to lose moisture to the surrounding breading. The decreased weight of shrimp coupled with an increased breading weight caused the product to fall below the United States Food and Drug Administration Standard of Identity for frozen breaded shrimp specifying a minimim shrimp content of 50% (7). Additional questions have been raised ragarding the nutritional and hedonic quality of breaded shrimp in the market place. Again breaded shrimp manufacturers believed that they were producing fine products, but the handling abuses at the retail level were depriving consumers of expected quality on some occasions. The following study was initiated to illuminate those problems.

The project was designed to determine chemical, microbiological, and organoleptic changes in frozen breaded shrimp as it moves through the wholesale-retail distribution system. A large Georgia seafood processor provided a single lot of 8-ounce frozen breaded fantail shrimp produced and blast frozen in Brownsville, Texas on July 6, 1982. The lot was trucked to a wholesale freezer in Glynn County, Georgia on July 11, 1982. Initial and replacement product was supplied from the freezer to two retail merchants operating in Brunswick, Georgia. The first market stored and displayed product for retail sale in a vertical or upright closed door freezer case, Warren/Sherer model HRL-5U. The second cooperator displayed and stored the frozen shrimp in a horizontal or coffin freezer open to the air, Hill model EZ 6KF. Product was loaded

into the upright freezer on July 29, 1982 and into the coffin freezer on August 10, 1982. Additional product was supplied to each retail store when it was requested by the manager. In all cases, product was delivered following completion of each month's sampling program.

METHODS

Ryan battery powered thermographs were used to continuously monitor temperatures at each storage location. A model K-45 recorder was installed in the warehouse freezer while a model K-10 thermograph was installed in each retail display case.

Five eight ounce packages were collected monthly from each storage area for chemical, microbiological, and organoleptic analyses. Samples from each location were composited for all determinations. The sample analyses of green headless shrimp collected before the Brownsville, Texas, production run were completed in July 1982. Frozen breaded shrimp collected from the wholesale freezer was first analyzed in July 1982. Initial retail samples of breaded shrimp from each display case were taken in August 1982. Monthly samples of frozen breaded shrimp from each storage location were collected through July 1983. The retail establishment providing the coffin freezer discontinued sales of seafood at the beginning of August 1983. Final breaded shrimp samples were collected from the upright freezer and the wholesale freezer in August 1983.

The following chemical analyses except for % breading were completed in duplicate for the initial green headless shrimp sample and monthly on breaded shrimp collected from each location:

(1) % moisture (9)
(2) % Kjeldahl Protein (9)
(3) % Ash (9)
(4) % Breading (9)
(5) Ammonium, specific ion electrode (12)
(6) Trimethylamine, specific ion electrode (2)

Bioassay vitamin analyses were completed for the same series of samples:

(1) Riboflavin (5,9)
(2) Thiamine (5,9)

Microbiological levels were determined for all green headless and frozen breaded shrimp samples:

(1) Aerobic Plate Count (8)
(2) MPN Total Coliforms and *E. coli* (8)
(3) MPN Coagulase Positive Staphylococci (8)

A taste panel consisting of 7 trained members evaluated frozen breaded shrimp samples fried for 3 minutes at 177°C in peanut oil for textural and flavor characteristics. A modified flavor profile was used to characterize each shrimp sample presented in duplicate to panel members. A continuous sensory scale of 0-5 was used to describe each flavor or textural characteristic. A score of zero indicated lack of detection by a panel member for a given trait while a score of five indicated the strongest impression of that trait (3). The following textural characteristics were defined:

(1) Hardness: The perceived force required to compress the sample using the molar teeth.
(2) Chewiness: The total perceived effort required to prepare the sample to a state ready for swallowing.
(3) Fibrousness: The perceived degree (number x size) of fibers evident during mastication.
(4) Oily Mouth Coating: The percieved degree of oil and/or water left on the teeth, tongue, and pallet after swallowing.
(5) Moistness: The perceived degree of oil and/or water in the sample during chewing.

The following flavor characteristics were defined:

(1) Overall Shrimp Intensity: The perceived degree of shrimp flavor exhibited by the sample.
(2) Sweet: The perceived degree of sweetness associated with the sample.
(3) Nutty Buttery: The aromatics associated with the rich full flavor of chopped nuts such as pecans and warm melted butter.
(4) Old Seafood: The aromatics associated with cooked seafood that is getting "off" but is still acceptable.
(5) Freezer Burn: The taste associated with a stale refrigerator or freezer that has been used to store food.
(6) Rancid Taste: The after taste common to country ham.

Panel members were presented with duplicate control samples of breaded shrimp at each meeting in addition to duplicates of the three experimental samples. The control sample was the latest code date breaded fantail shrimp produced by a local seafood processor.

RESULTS AND DISCUSSION

A. Temperature

The warehouse freezer exhibited maximum daily temperature variations of 2 - 3°C, the smallest deviation of any freezer studied (Figure 1). Most monitored temperatures were below -20°C. The maximum recorded temperature of -10°C occurred in August 1982 following a freezer malfunction. The minimum temperature of -24°C occurred during December 1982 and January 1983. No consistent defrost cycle was noted.

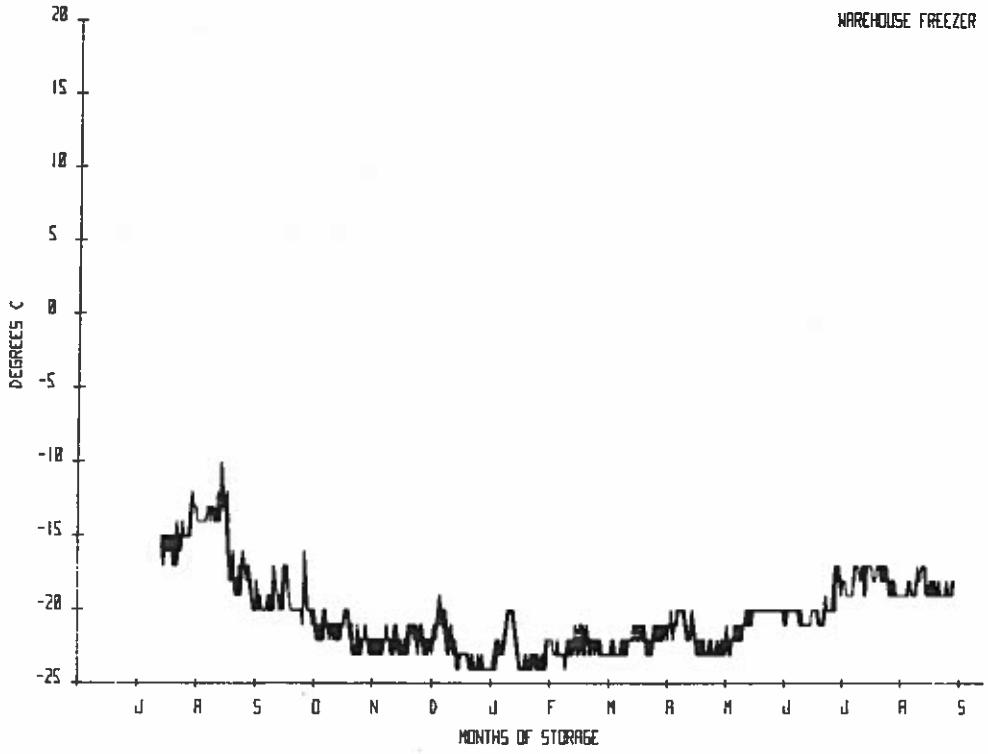

Figure 1. Mean daily maximum and minimum storage temperatures and mean temperature variations for the warehouse freezer.

A maximum daily temperature variation of 34°C occurred for the coffin freezer in February 1983 (Figure 2). Normal daily temperature variation was approximately 12°C with a 6 - 8 hour defrost cycle. The freezer remained below -7°C for most of the study. The coffin freezer reached a maximum temperature of 17°C following malfunctions in November 1982 and January 1983. The minimum temperature of -20°C was achieved in February and March 1983.

The upright freezer had a maximum daily temperature variation of 25°C in October 1982 (Figure 3). A general 6 - 12 hour defrost cycle with a 14 - 18°C temperature variation was observed for the freezer. Product temperature remained below -7°C for most of the study. The freezer reached a maximum temperature of 3°C in October 1982 and a minimum temperature of -24°C in both October and November 1982.

The minimum daily freezer temperatures observed for the warehouse freezer were consistently less than those measured for the upright freezer (Figure 4). The mean minimum daily coffin freezer temperatures were consistently greater than those of the warehouse or upright freezer. The maximum daily temperatures of the coffin freezer were greater than those of the upright freezer which in turn were greater than the warehouse freezer temperatures (Figure 5).

B. Analyses

Table 1 presents the dates that frozen breaded shrimp were delivered from the warehouse freezer to the retail, upright, and coffin freezers.

Coffin Freezer	Upright Freezer
	29 July 1982
10 August 1982	25 August 1982
19 October 1982	
	12 November 1982
14 December 1982	
2 March 1983	23 March 1983
27 April 1983	27 April 1983
6 July 1983	6 July 1983

Table 1. Product delivery dates to the retail freezers.

Tables 2 - 4 present the chemical, vitamin, and microbiological analyses of the frozen green headless shrimp used to produce the control lot of breaded shrimp at the Brownsville facility. Figures 6 - 33 detail the results of monthly chemical, microbiological, and organoleptic analyses of the frozen breaded shrimp stored in each of the freezers.

Table 2. Mean chemical analyses of green headless shrimp used to produce the control lot of frozen breaded shrimp.

% Moisture	% Protein	% Ash	Ammonium mg/100g	TMA mg/100g
74.78	18.48	4.39	18.0	7.98

All chemical, microbiological and organoleptic data sets containing duplicate or greater than two values for each dependent variable were analyzed statistically with the Statistical Analytical System (SAS) (11). The methods included the General Linear Regerssion Model (GLM) utilizing an analysis of variance procedure to compare monthly data for each dependent variable at each storage location. Dependent variable means were compared for significant differences at the 0.05 level using Tukey's studentized range test (HSD) (11). In the remainder of the paper, a significant difference between means refer to Tukey's studentized range test with $F > 0.05$.

Of the chemical analyses: net weight (Figure 6), percent moisture (Figure 7), and ammonium (Figures 8 and 9) levels were most useful in differentiating the three treatment conditions. The wholesale product

Table 3. Mean vitamin content of green headless shrimp used to produce the control lot of frozen breaded shrimp.

Thiamine mg/100g	Riboflavin mg/100g
0.050	0.003

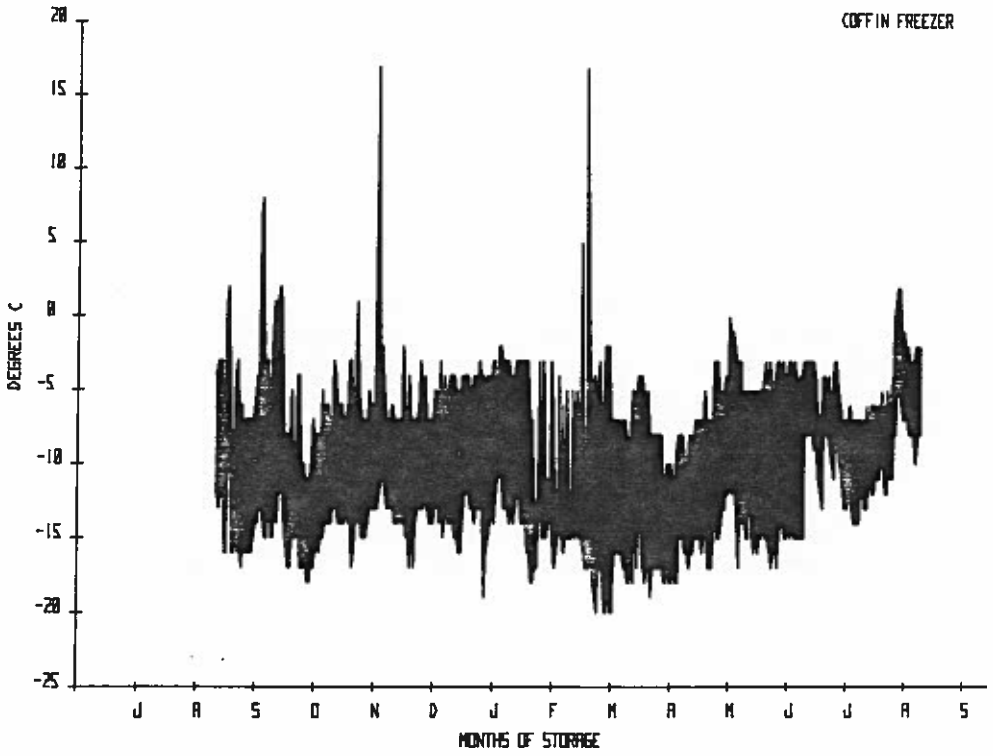

Figure 2. Mean daily maximum and minimum storage temperatures and mean temperature variations for the coffin freezer.

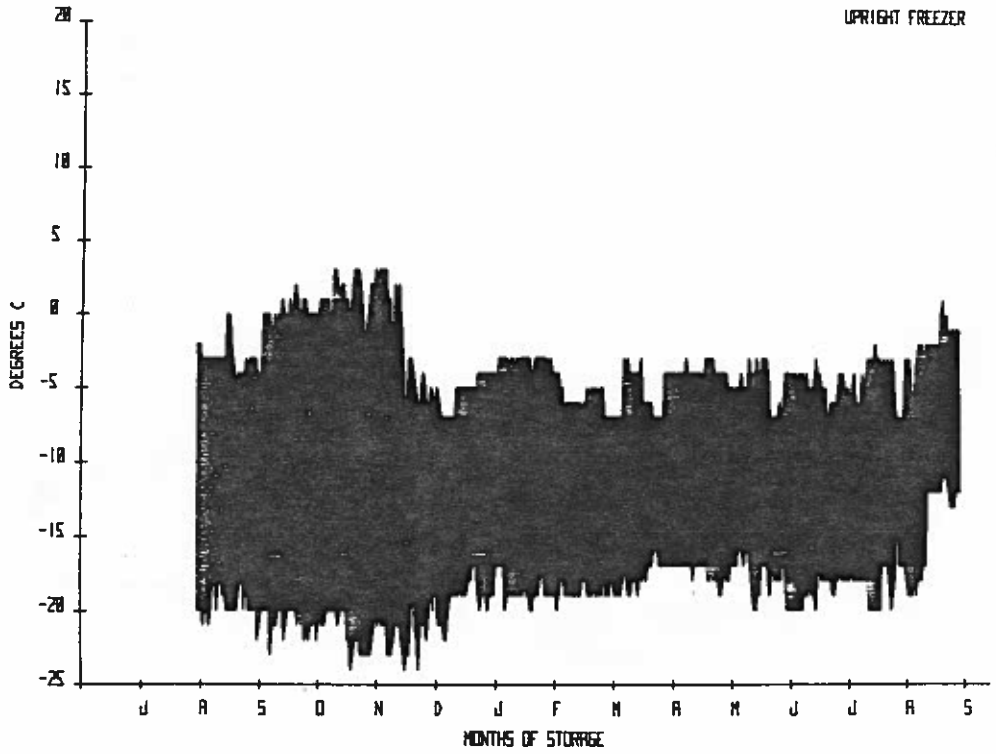

Figure 3. Mean daily maximum and minimum storage temperatures and mean temperature variations for the upright freezer.

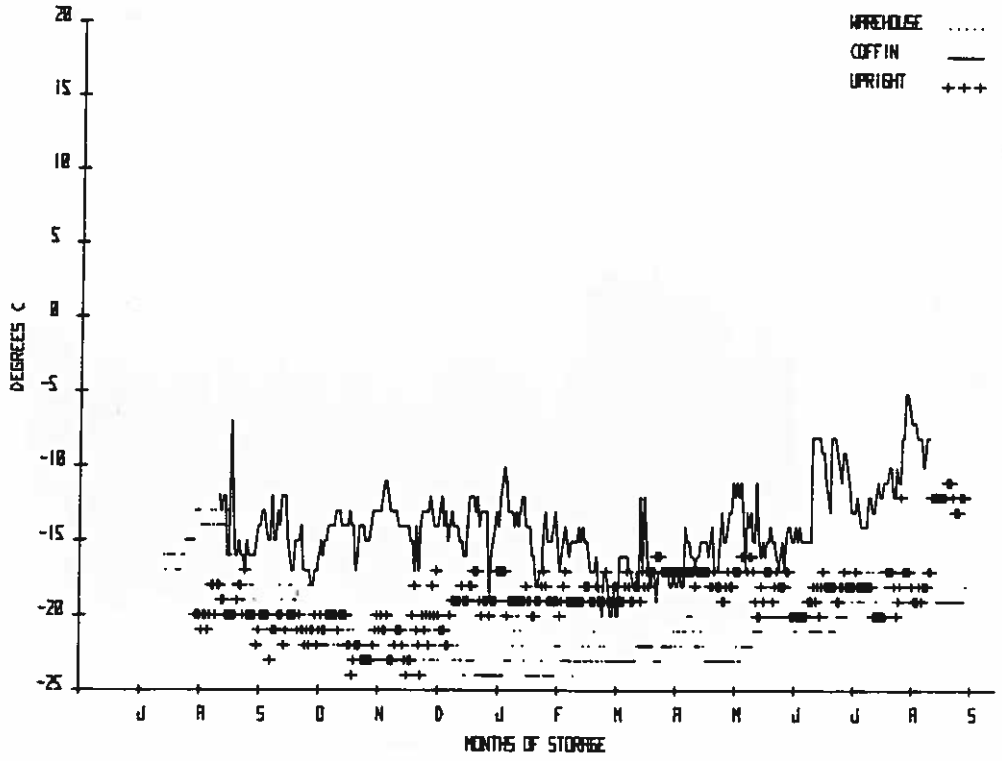

Figure 4. The mean minimum daily temperatures for the warehouse, coffin, and upright freezers.

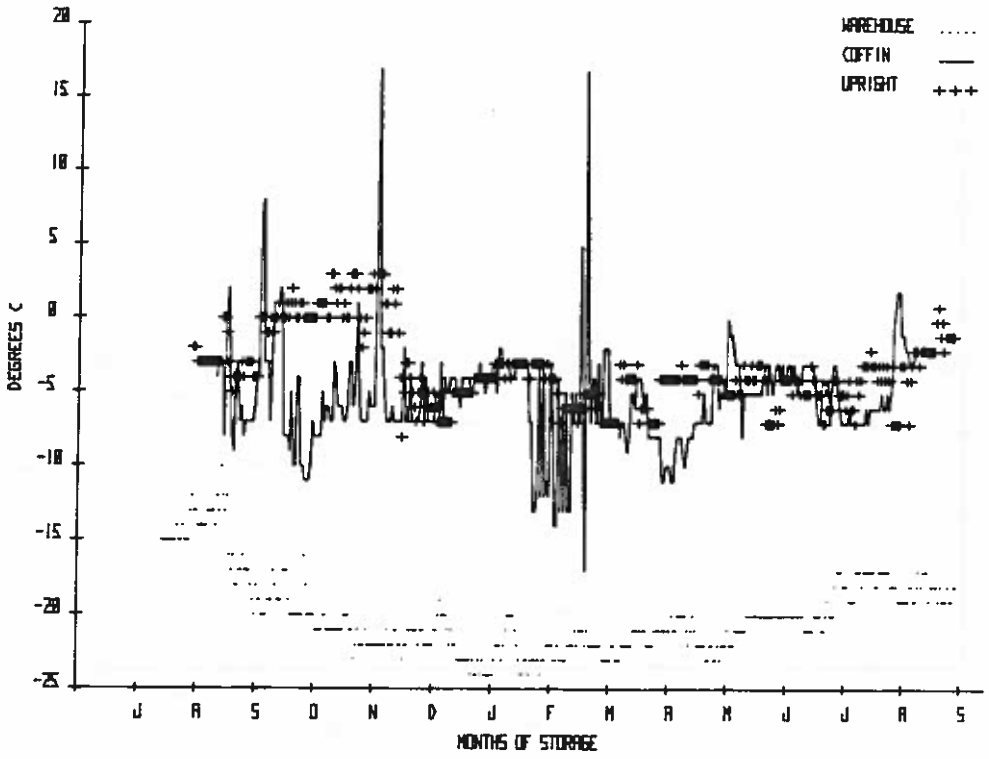

Figure 5. The mean maximum daily temperatures for the warehouse, coffin, and upright freezers.

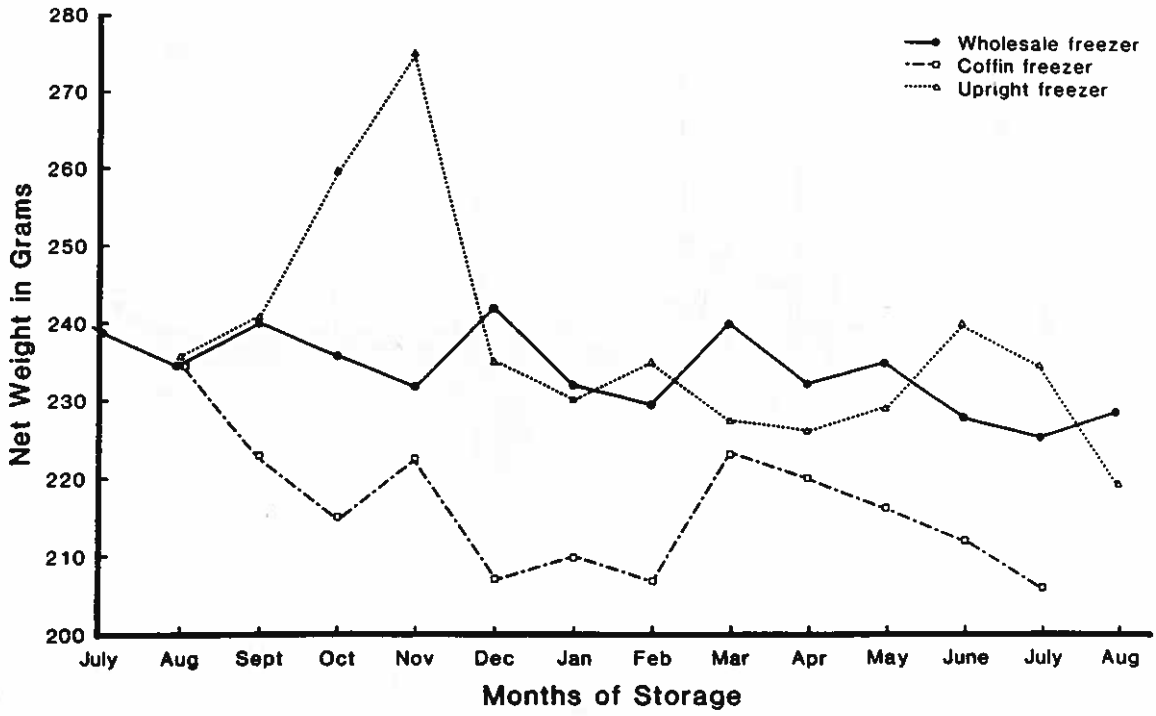

Figure 6. Mean monthly net weights in grams of shrimp held in the wholesale, coffin, and upright freezers.

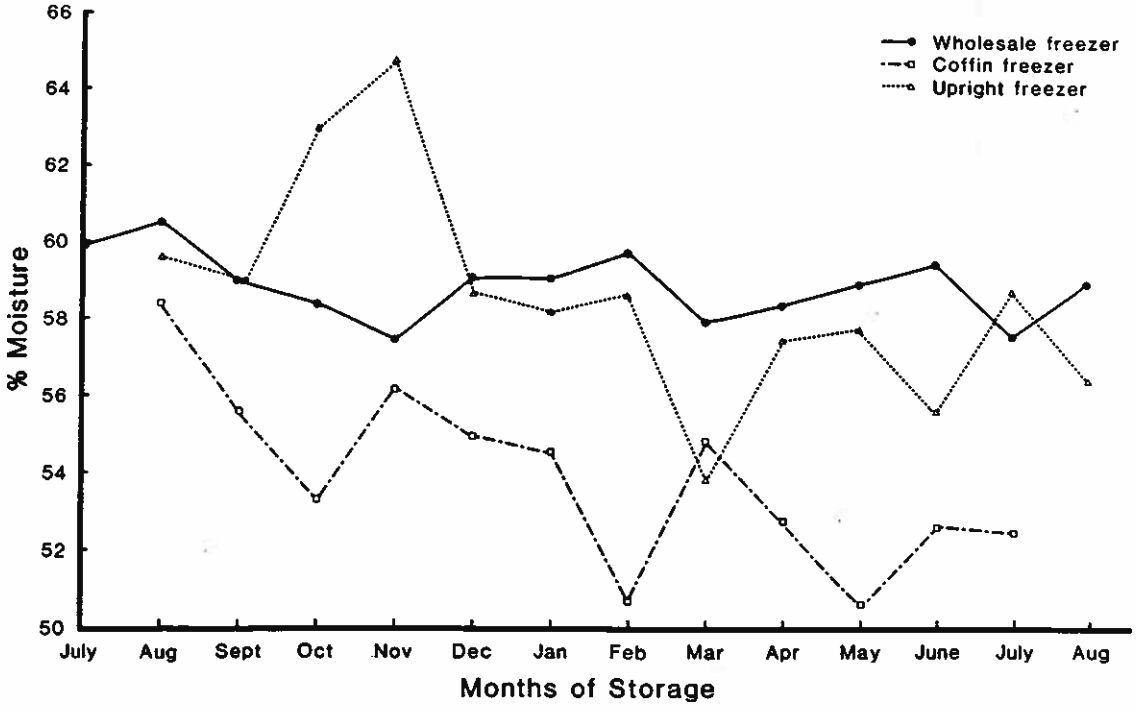

Figure 7. Mean monthly % moisture content of breaded shrimp stored in the wholesale, coffin and upright freezers.

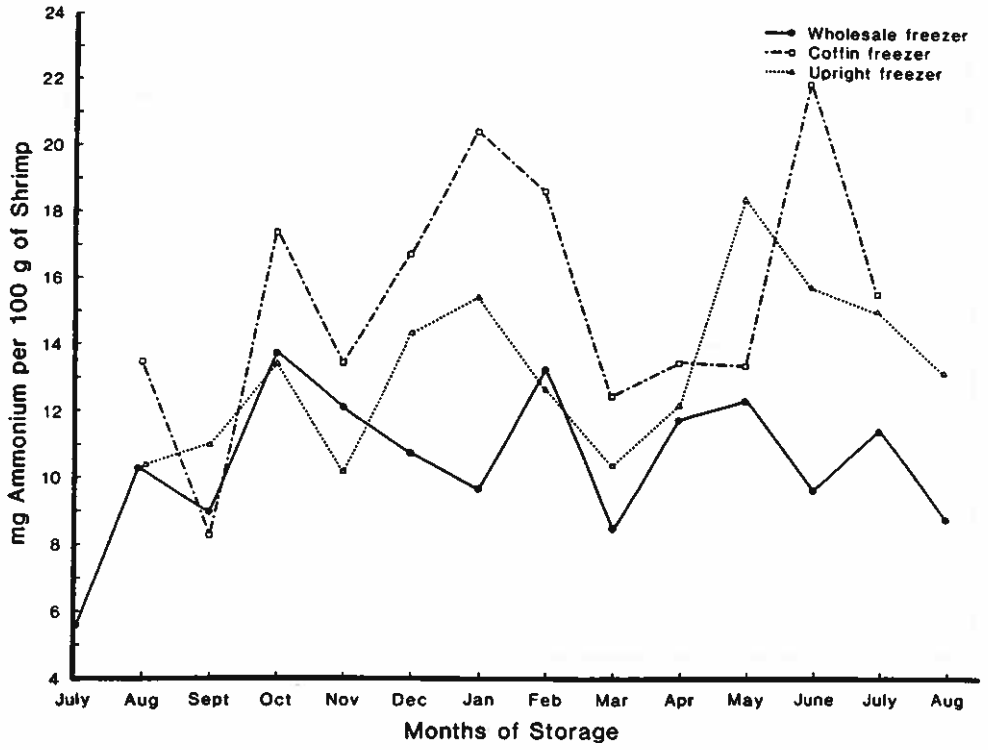

Figure 8. Mean monthly ammonium levels in breaded shrimp stored in the wholesale, coffin, and upright freezers.

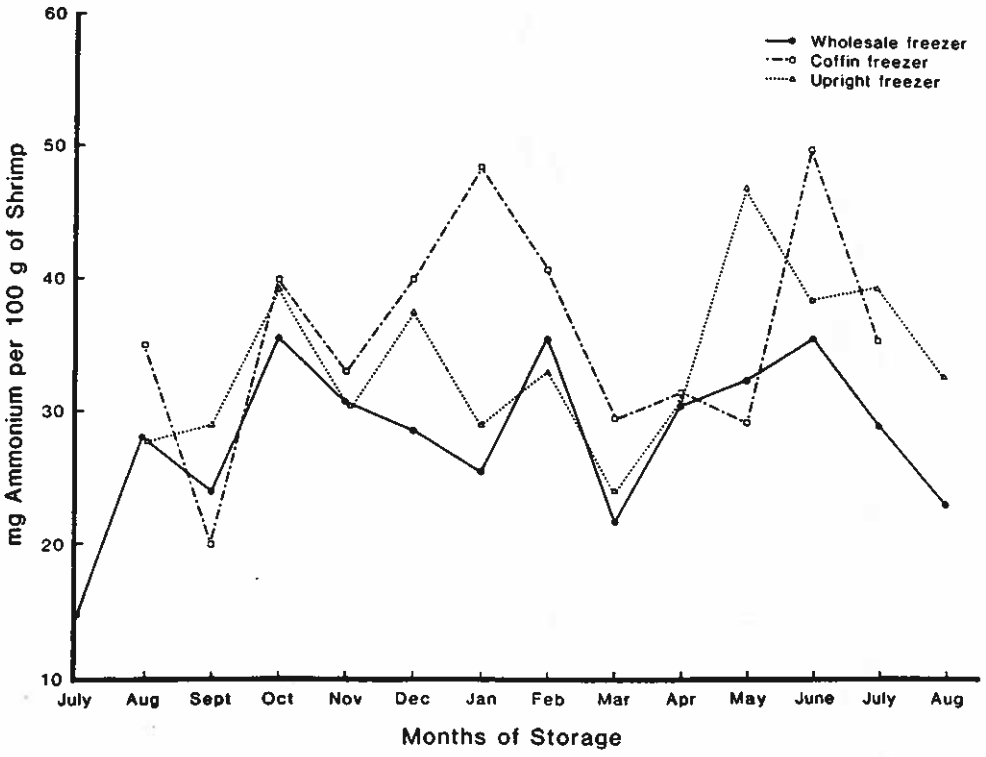

Figure 9. Mean monthly free/ash free ammonium levels in breaded shrimp stored in the wholesale, coffin, and upright freezers.

Aerobic Plate Count Organisms/g	MPN Total Coliforms Organisms/g	MPN *E. coli* Organisms/g	MPN Coagulase Pos. Staph. Organisms/g
2.40×10^5	240	<2	<3

Table 4. Mean microbiological levels of green headless shrimp used to produce the control lot for frozen breaded shrimp.

lost 4.31% net weight by the end of the storage study (Table 5). The mean net weight of product stored in the upright freezer increased 8.72% and 15.33% respectively, in October and November (1982) but registered a net decrease of 5.45% by the end of the study. Weight loss in the coffin freezer was greatest, with a net decrease of 13.44% when the study terminated. Moisture values reflected the net weight data (Table 6). The wholesale freezer lost 0.03% moisture, the upright freezer 2.52% and the coffin freezer 6.49%. Percent moisture increases of 4.10% and 5.85% in October and November 1982 parallelled net weight gains in the upright freezer during the same months. Maximum ammonium levels at the beginning and at the end of the study exhibited a similar pattern: wholesale freezer 5.60 mg/100g - 8.75 mg/100g, upright freezer 10.4 mg/100g - 13.2 mg/100g, and coffin freezer 13.5 mg/100g - 15.6 mg/100g. Trimethylamine data failed to differentiate the three treatment conditions (Figures 10 and 11, Tables 7 and 8).

Tukey's studentized range test applied to the chemical data indicated that net weights (Table 9) and percent moisture (Table 10), followed by ammonium levels (Tables 11 and 12) were the most effective analyses to differentiate product collected from the three storage areas. The mean net weights of the coffin freezer samples were significantly less than those of the upright and/or warehouse freezer samples from the third through the twelfth month of storage with the exception of the eighth and tenth months (Table 9). The mean net weights of the upright freezer samples were significantly greater than the other samples in October and November 1983, concurrent with observed ice crystal formation and increased maximum daily temperatures of the upright freezer. Moisture levels determined for the coffin freezer shrimp were significantly less than the other storage area samples from one month of storage until the end of the project, with the exception of the ninth month. The upright freezer samples registered significantly greater % moisture content in October and November, parallelling the net weight gains for the same months (Table 10). Product dessication was indicated by significantly lower moisture levels determined for upright samples during 6 of the last 9 months of storage, including the final August 1983 sample. Ammonium levels from coffin stored shrimp were significantly greater than the other sample values from 5 months of storage through the eleventh month except for the ninth month (Table 11). The freezer was restocked one month before the sample was collected.

The percent shrimp (Figure 12) determined for all wholesale and retail samples failed to meet the Standard of Identity for breaded shrimp that specifies a minimum content of 50% shrimp for frozen raw breaded shrimp (7). The initial wholesale freezer sample, 6 days after the production run, contained 5.26% less shrimp (46.24%) than was determined at the Brownsville, Texas facility (51.50%) on the production line (Table 13). The rapid decrease in percent shrimp, net weight decreases, and percent moisture changes attributed to moisture migration from the shrimp into the surrounding breading and out of the package are consistent with studies by Rao (10) and Williams (14).

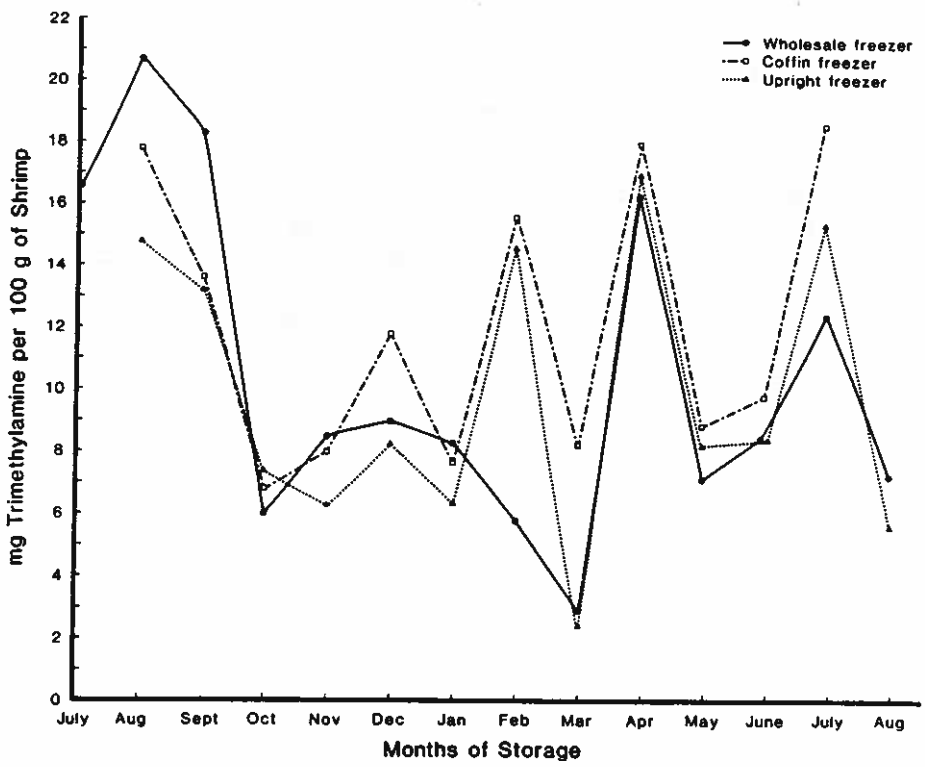

Figure 10. Mean monthly trimethylamine levels for the wholesale, coffin, and upright freezers.

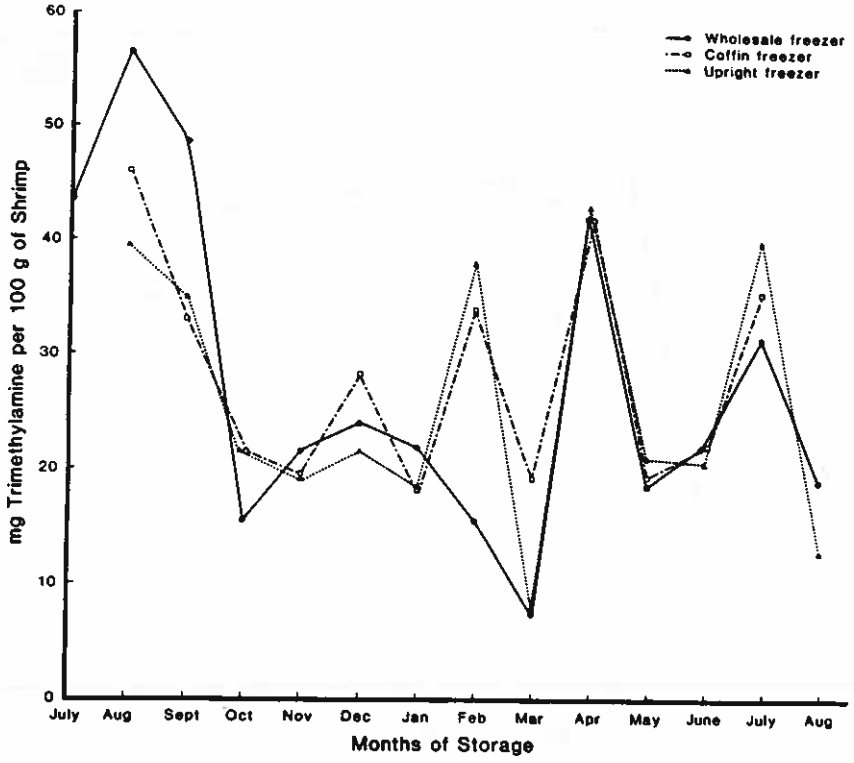

Figure 11. Mean monthly moisture free/ash free trimethylamine levels for the wholesale, coffin, and upright freezers.

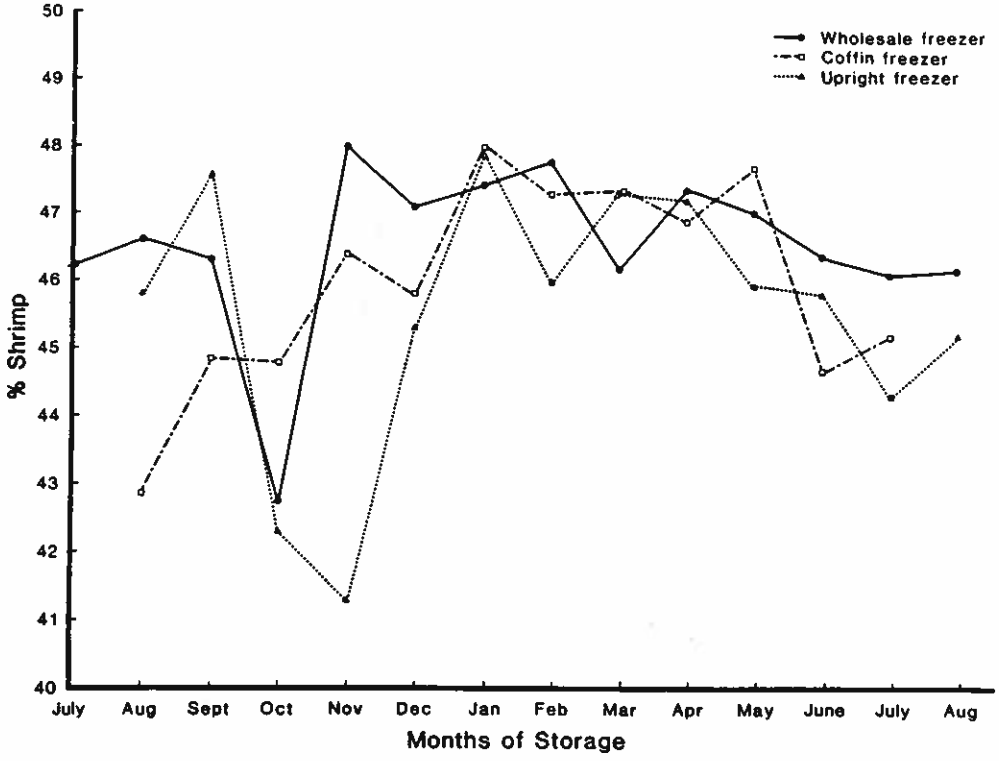

Figure 12. Monthly % shrimp for the wholesale, coffin and upright freezers.

Storage Time	Wholesale Freezer	Retail Coffin Freezer	Retail Vertical Freezer
2 weeks	238.76(0.00%)	----------	----------
1 month	234.49(-1.79%)	234.58(-1.75%)	235.65(-1.30%)
6 months	232.09(-2.79%)	209.67(-12.18%)	233.17(-2.34%)
9 months	232.35(-2.68%)	220.03(-7.84%)	226.33(-5.21%)
12 months	225.37(-5.61%)	206.68(-13.44%)	234.58(-1.75%)
13 months	228.47(-4.31%)	----------	225.75(-5.45%)

Table 5. Mean net weight values of frozen breaded shrimp and percent change from the initial wholesale freezer mean value.

Storage Time	Wholesale Freezer	Retail Coffin Freezer	Retail Vertical Freezer
2 weeks	58.96%(0.00%)	----------	----------
1 month	60.55%(+1.59%)	58.43%(-0.53%)	59.63%(+0.67%)
6 months	59.06%(+0.10%)	54.29%(-4.67%)	57.23%(-1.73%)
9 months	58.45%(-0.51%)	53.71%(-5.25%)	57.49%(-1.47%)
12 months	57.58%(-1.38%)	52.47%(-6.49%)	58.74%(-0.22%)
13 months	58.93%(-0.03%)	----------	56.44%(-2.52%)

Table 6. Mean percent moisture values and percent change of frozen breaded shrimp calculated from the initial wholesale freezer mean value.

TRIMETHYLAMINE
mg/100g

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		March 83	A 8.16	Coffin
				B 3.42	Upright
Aug 82	NSD			B 2.90	Warehouse
Sept 82	NSD		April 83	NSD	
Oct 82	NSD		May 83	NSD	
Nov 82	NSD		June 83	NSD	
Dec 82	NSD		July 83	A 18.62	Coffin
				B 15.37	Upright
Jan 83	NSD			B 12.41	Warehouse
Feb 83	A 15.60	Coffin	Aug 83	NSD	
	A 14.62	Upright			
	B 5.79	Warehouse			

NSD = No significant difference

Table 7. Mean trimethylamine mg/100g shrimp significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

MOISTURE FREE/ASH FREE TRIMETHYLAMINE
mg/100g

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 40.22	Upright
				A 33.91	Coffin
Aug 82	A 56.54	Warehouse		B 15.50	Warehouse
	A 47.42	Coffin			
	B 14.78	Upright	March 83	A 19.38	Coffin
				B 7.96	Upright
Sept 82	NSD			C 7.41	Warehouse
Oct 82	A 21.04	Upright	April 83	NSD	
	B 15.52	Coffin			
	B 15.46	Warehouse	May 83	NSD	
Nov 82	NSD		June 83	NSD	
Dec 82	A 26.81	Coffin	July 83	A 43.13	Coffin
	BA 23.89	Warehouse		A 40.05	Upright
	B 21.28	Upright		B 31.45	Warehouse
Jan 83	NSD		Aug 83	A 19.72	Warehouse
				B 13.78	Upright

NSD = No significant difference

Table 8. Mean moisture free/ash free trimethylamine mg/100g shrimp significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

NET WEIGHT GRAMS

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 235.10	Upright
				A 229.49	Warehouse
Aug 82	NSD			B 206.71	Coffin
Sept 82	A 240.63	Upright	March 83	NSD	
	A 240.17	Warehouse			
	B 222.08	Coffin	April 83	A 232.35	Warehouse
				BA 226.33	Upright
Oct 82	A 259.59	Upright		B 220.03	Coffin
	B 235.58	Warehouse			
	C 215.16	Coffin	May 83	NSD	
Nov 82	A 275.36	Upright	June 83	A 239.88	Upright
	B 231.74	Warehouse		BA 227.96	Warehouse
	B 222.55	Coffin		B 211.85	Coffin
Dec 82	A 241.96	Warehouse	July 83	A 234.58	Upright
	A 235.10	Upright		BA 225.37	Warehouse
	B 207.06	Coffin		B 206.68	Coffin
Jan 83	A 232.09	Warehouse	Aug 83	NSD	
	BA 230.30	Upright			
	B 209.67	Coffin			

NSD = No significant difference

Table 9. Mean net weights significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

% MOISTURE

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 59.76	Warehouse
				A 58.68	Upright
Aug 82	A 60.55	Warehouse		B 50.70	Coffin
	B 59.63	Upright			
	C 58.43	Coffin	March 83	A 57.94	Warehouse
				B 54.86	Coffin
Sept 82	A 59.22	Warehouse		B 53.89	Upright
	A 59.10	Upright			
	B 55.64	Coffin	April 83	NSD	
Oct 82	A 63.06	Upright	May 83	A 58.92	Warehouse
	B 58.40	Warehouse		B 57.84	Upright
	C 53.35	Coffin		C 50.64	Coffin
Nov 82	A 64.30	Upright	June 83	A 59.49	Warehouse
	B 57.50	Warehouse		B 55.62	Upright
	B 56.21	Coffin		C 52.65	Coffin
Dec 82	A 59.57	Warehouse	July 83	A 58.74	Upright
	B 58.70	Upright		A 57.58	Warehouse
	C 54.95	Coffin		B 52.47	Coffin
Jan 83	A 59.09	Warehouse	Aug 83	A 58.92	Warehouse
	B 57.22	Upright		B 56.44	Upright
	C 54.54	Coffin			

NSD = No significant difference

Table 10. Mean % moisture significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

AMMONIUM
mg/100g

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 18.75	Coffin
				B 13.25	Warehouse
Aug 82	NSD			B 12.65	Upright
Sept 82	A 11.00	Upright	March 83	A 12.50	Coffin
	BA 9.00	Warehouse		BA 10.40	Upright
	B 8.30	Coffin		B 8.50	Warehouse
Oct 82	A 17.45	Coffin	April 83	NSD	
	B 13.75	Warehouse			
	B 13.50	Upright	May 83	A 18.50	Upright
				B 13.45	Coffin
Nov 82	A 13.50	Coffin		B 12.35	Warehouse
	BA 12.15	Warehouse			
	B 10.25	Upright	June 83	A 22.00	Coffin
				B 15.85	Upright
Dec 82	A 16.75	Coffin		B 9.65	Warehouse
	A 14.40	Upright			
	B 10.75	Warehouse	July 83	NSD	
Jan 83	A 20.50	Coffin	Aug 83	A 13.20	Upright
	A 15.50	Upright		B 8.75	Warehouse
	B 9.70	Warehouse			

NSD = No significant difference

Table 11. Mean ammonium levels, mg/100g shrimp, significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

MOISTURE FREE/ASH FREE AMMONIUM
mg/100g

Month	Mean	Freezer	Month	Mean	Freezer
July 82	NSD		March 83	A 29.48	Coffin
				BA 24.18	Upright
Aug 82	NSD			B 21.72	Warehouse
Sept 82	A 28.94	Upright	April 83	NSD	
	BA 23.90	Warehouse			
	A 20.12	Coffin	May 83	A 47.13	Upright
				B 32.30	Warehouse
Oct 82	NSD			B 29.24	Coffin
Nov 82	NSD		June 83	A 50.00	Coffin
				B 38.49	Upright
Dec 82	A 39.90	Coffin		B 25.60	Warehouse
	A 37.56	Upright			
	B 28.58	Warehouse	July 83	NSD	
Jan 83	A 48.60	Coffin	Aug 83	A 32.75	Upright
	BA 39.10	Upright		B 23.00	Warehouse
	B 25.54	Warehouse			
Feb 82	NSD				

NSD = No significant difference

Table 12. Mean moisture free/ash free ammonium levels, mg/100g shrimp, significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

Storage Time	Wholesale Freezer	Retail Coffin Freezer	Retail Vertical Freezer
0	51.50%(0.00%)*	----------	----------
2 weeks	46.24%(-5.26%)	----------	----------
1 month	45.81%(-5.62%)	42.86%(-8.64%)	45.81%(-5.69%)
6 months	47.43%(-4.07%)	48.04%(-3.46%)	47.88%(-3.62%)
9 months	47.36%(-4.14%)	46.87%(-4.63%)	47.20%(-4.30%)
12 months	46.12%(-5.38%)	45.18%(-6.32%)	44.32%(-7.18%)
13 months	46.17%(-5.33%)	----------	45.20%(-6.30%)

* Before freezing

Table 13. Mean percent shrimp values and percent change of frozen breaded shrimp calculated from the initial percent shrimp content on the production line before freezing.

Williams monitored changes in net weight, percent moisture, and percent shrimp during a nine month study of blast frozen breaded shrimp stored in warehouse and retail freezers. Williams' results were similar to those determined by this study (14). The net weight of shrimp stored in the retail freezer decreased more rapidly than shrimp stored in the warehouse freezer (Table 14). The mean net weight of shrimp frozen for Williams study decreased 0.6% following freezing by the blast freezer. After freezing, percent decreases for the wholesale freezer at one, six, and nine months were 1.3%, 0.2%, and 1.8% compared with 1.79%, 2.79%, and 2.68% decreases for the present study (Table 5). Williams' retail freezer samples lost 1.4%, 6.2%, and 9.3% net weight following one, six, and nine months of storage. The vertical freezer samples lost less weight than Williams' retail freezer with 1.30%, 2.34%, and 5.21% decreases while the coffin freezer results were mixed with 1.75%, 12.18%, and 7.84% decreases for one, six, and nine months, respectively. Percent moisture changes detected from shrimp stored in Williams' wholesale and retail freezers were similar to the present results. Initial freezing reduced mean moisture content by 0.9%. Moisture loss was accelerated in his retail freezers, compared with the warehouse freezers. Moisture loss from samples in Williams' warehouse freezer was greater than that determined for the present study. The dessication of samples from Williams' retail freezer was greater than that determined for the upright freezer but less than the observed values for the coffin freezer (Tables 6 and 14).

Table 14. Mean percent net weight and moisture changes in blast frozen breaded shrimp calculated from data presented by S.K. Williams, et al. (1981).

Storage Time	Net Weight		Moisture	
	Wholesale	Retail	Wholesale	Retail
2 weeks	-1.1%	-1.2%	+0.8%	0.0%
1 month	-1.3%	-1.4%	+0.7%	+0.5%
6 months	-0.2%	-6.2%	-1.4%	-3.6%
9 months	-1.8%	-9.3%	-1.2%	-6.3%

Data from Rao (10) and Williams (14) indicated a rapid loss in the relative percent shrimp present in a breaded shrimp sample after freezing. Williams' samples lost 6.80% shrimp following blast freezing which reduced mean percent shrimp levels from 51.1% to 44.3%. The relative percent loss of shrimp was reduced to 4.3% by the second week (Table 15). The current study determined a 5.26% shrimp loss at the end of two weeks storage in the warehouse freezer (Table 13). Rao reported 14.24% and 13.62% losses following one and two weeks of storage (Table 16). The overall decrease in percent shrimp was greater for the retail freezers than the warehouse freezers in Williams' and the present study (Tables 13 and 15). In the current study percent shrimp loss decreased for both wholesale and retail freezers, after initial freezing, reached a minimum at six months and increased through the next six months of storage (Table 13, Figure 12).

The transfer of moisture to and from packaged breaded shrimp and the movement of moisture within the product itself represents a continuing quality and regulatory problem facing the manufacturers of frozen breaded shrimp. The data indicated that the storage of breaded shrimp at the retail level compounds the problem and hastens quality deterioration. The constant defrosting and higher storage temperatures of the coffin and upright freezers reduced moisture contents, net weights, and percent shrimp of products stored at the retail level more rapidly than was observed under the controlled conditions encountered in the wholesale freezer. The higher mean temperatures observed in the coffin freezer produced significant reductions in the mean net weights of coffin samples by the second month of storage and significant reductions in moisture content by the first month of storage. The upright freezer samples experienced a significant net weight gain in October and November of 1982. Large ice crystals collected in the packages following two months of freezer defrost temperatures that exceeded 0°C. Significant dessication was also observed in the upright freezer by the end of the study. The development of frozen seafood packaging that provides a moisture barrier and a program to educate retail merchants on proper frozen seafood handling and temperature control would reduce the dessication of frozen breaded products. The control of moisture migration within the product was beyond the scope of this project; however, the work of Williams (14) and Rao (10), provided some answers to moisture migration problems associated with frozen breaded shrimp. The use of rapid IQF (individually quick frozen) processes and finer breading material helped reduce percent shrimp loss from frozen product. Moisture loss was less rapid and net weights remained more constant following rapid freezing. Fine breading helped maintain a more constant equilibrium between percent breading and percent shrimp values. The fine breading wicked away moisture at a rate more closely approximating the migration of moisture from the shrimp into the breading.

The nutritional and microbiological analyses revealed no apparent differences for breaded shrimp stored in the three freezers. Product wet

Storage Time	Percent shrimp	
	Wholesale	Retail
2 weeks	-4.3%	-3.2%
1 month	-5.6%	-2.7%
6 months	-6.0%	-5.3%
9 months	-5.7%	-7.9%

Table 15. Mean percent shrimp changes in blast frozen breaded shrimp calculated from data presented by S.K. Williams, et al. (1981).

Storage Time Weeks	Percent shrimp
1	-14.24
2	-13.62
3	-13.35
4	-11.84
5	-10.11

Table 16. Mean percent shrimp changes in slow frozen (-21°C) breaded shrimp from Rao et al (1975).

weight protein (Figure 13) and ash (Figure 14) increased from April through July in the coffin freezer, but the trend disappeared on moisture free/ash free (Figure 15) and moisture free bases, respectively (Tables 17, 18, and 19). Thiamine levels decreased rapidly for all three experimental samples from July to September, 1982. Thiamine concentrations (Figures 16 and 17) stabilized and randomly ranged from 0.014 - 0.043 mg/100g shrimp with a mean of 0.025 mg/100g for the remainder of the study. Riboflavin concentrations decreased from their initial levels, but stabilized in November 1982 and randomly ranged from 0.016 - 0.057 mg/100g shrimp with a mean of 0.037 mg/100g through August 1983 (Figures 18 and 19). The range of thiamine concentrations from September 1982 (0.014 - 0.043 mg/100g) bracketed the 0.03 mg/100g value given by the Department of Argiculture Handbook No. 8 (13) for breaded shrimp. The mean value 0.025 mg/100g was less than the accepted value. Riboflavin levels from November 1982 (0.016 - 0.057 mg/100g) bracketed the Department of Agriculture value of 0.03 mg/100g and the mean of 0.037 mg/100g exceeded the published value. All microbiological levels were within Georgia Department of Agriculture standards (Figures 20, 21, and 22). No sample differences between means were noted for the aerobic plate counts (Table 20).

The nutritional quality of shrimp stored in the wholesale and retail freezers was maintained throughout the study as evidenced by protein, thiamine, and riboflavin levels. The nutritional quality of the shrimp could not be differentiated by the three storage conditions. Thiamine and riboflavin concentrations decreased following the first 3 - 4 months of storage, but the mean vitamin levels were within -0.005 mg/100g and +0.007 mg/100g of published vitamin levels for thiamine and riboflavin, respectively.

The textural and flavor characteristics of shrimp stored in the wholesale and retail freezers, as perceived by the taste panel, proved to be the most discriminating tool to differentiate product quality throughout the study. Hardness (Figure 23) and chewiness (Figure 24) increased for both coffin and upright freezer samples with time. The initial and final horizontal and vertical ratings were 2.29 - 4.11, 2.14 - 2.75, hardness and 2.36 - 4.21, 2.29 - 2.75 chewiness. Hardness and chewiness ratings of coffin freezer samples exceeded both upright and wholesale levels by November 1982 and the upright freezer exceeded wholesale levels in June 1983. Wholesale freezer hardness and chewiness levels decreased from 3.00 - 2.00 and 3.14 - 2.07, respectively. The coffin freezer samples were rated significantly harder than the other samples by the fourth month of storage. The pattern continued until the end of the study, except for March 1983 which showed no significant difference between upright and horizontal samples (Table 21). The upright freezer had not been restocked for four months before the March sample was collected. The chewiness pattern was similar to the hardness observations for the coffin freezer. The upright freezer samples were significantly chewier than the warehouse samples for the twelfth and thirteenth months of storage (Table 22). Coffin freezer fibrouness (Figure 25)

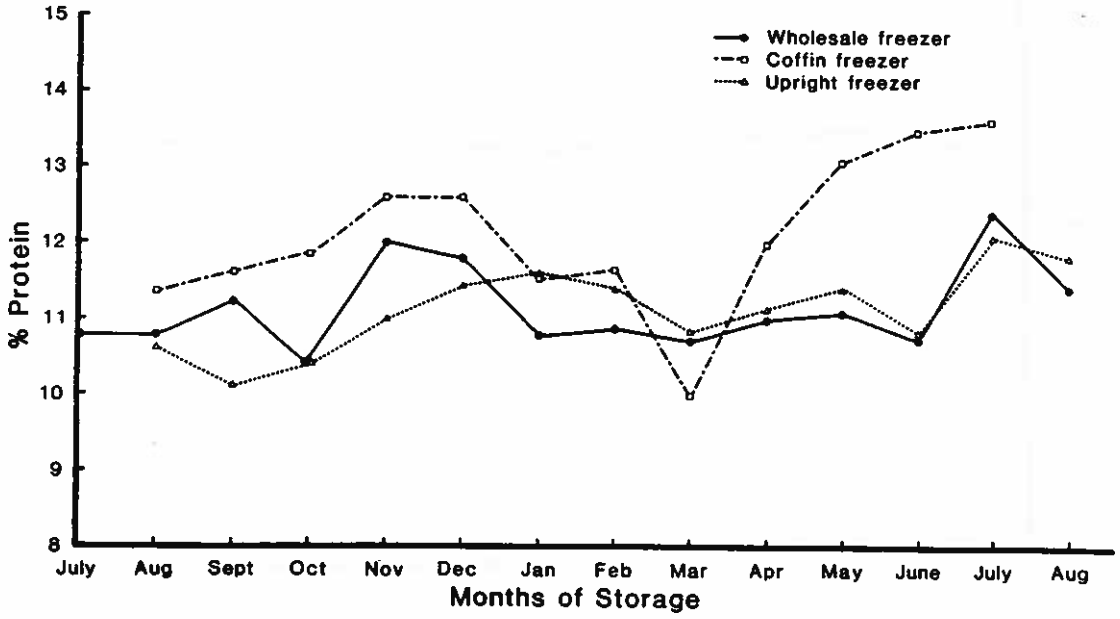

Figure 13. Mean monthly % protein levels in the wholesale, coffin, and upright freezers.

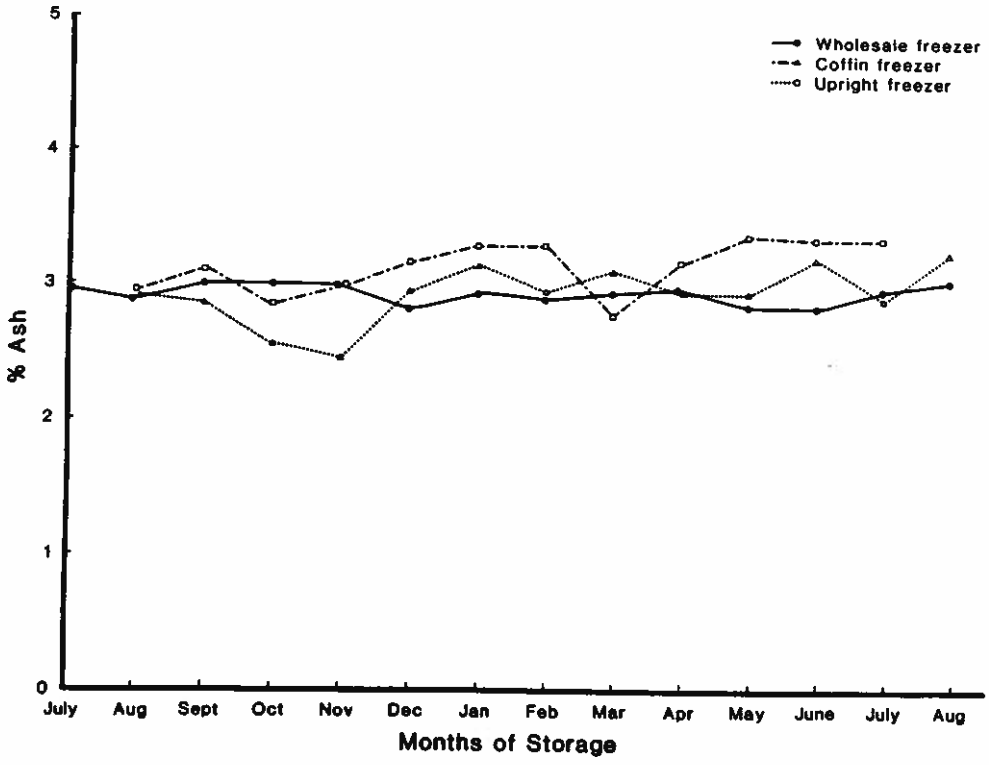

Figure 14. Mean monthly % ash levels for the wholesale, coffin, and upright freezers.

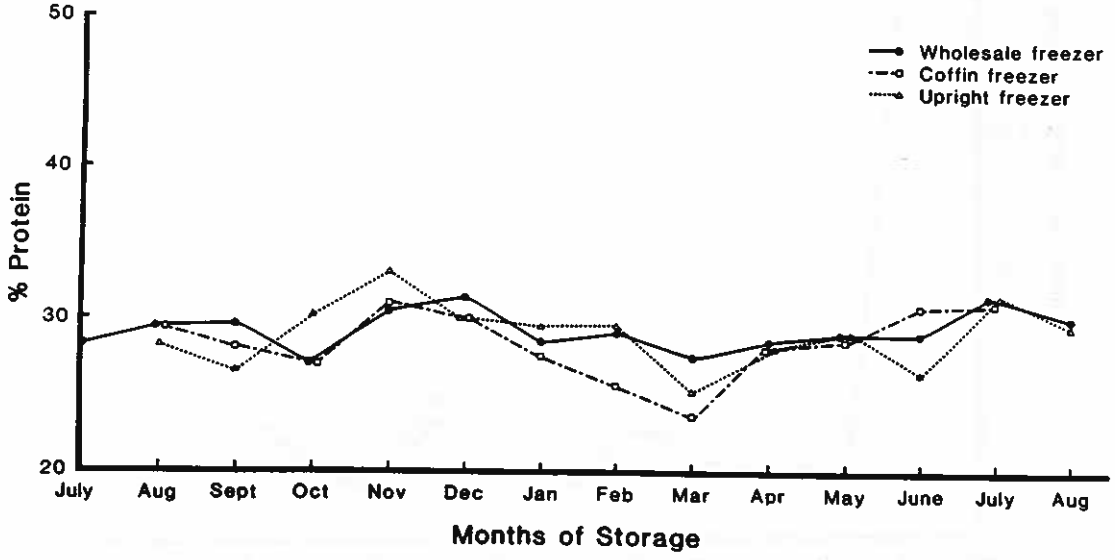

Figure 15. Mean monthly moisture free/ash free % protein levels in the wholesale, coffin, and upright freezers.

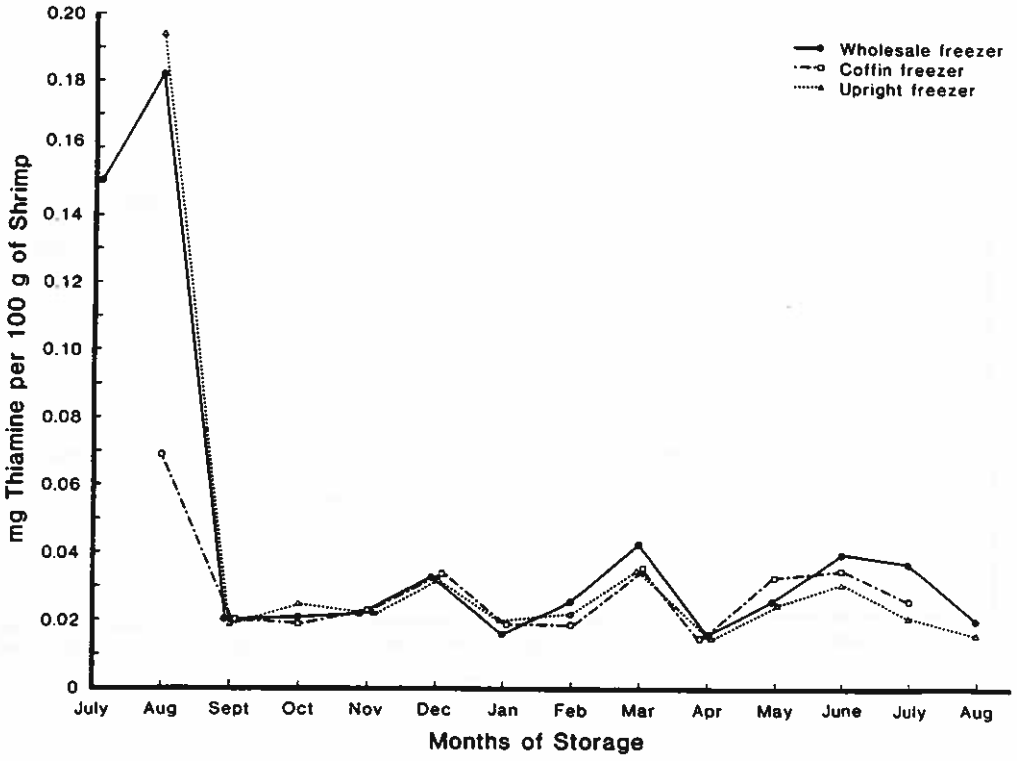

Figure 16. Monthly thiamine concentrations for the wholesale, coffin, and upright freezers.

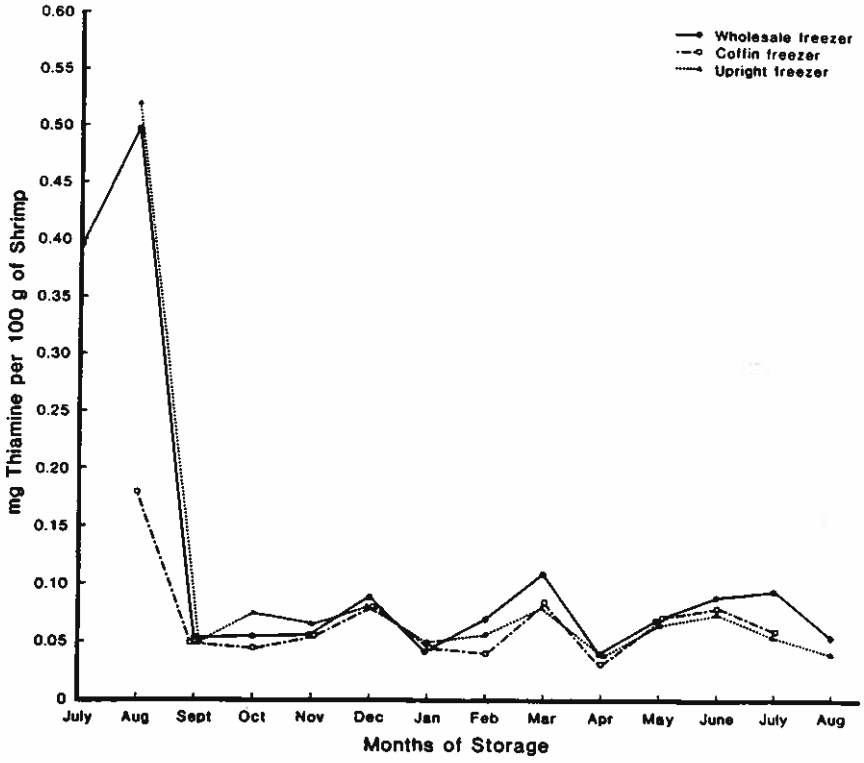

Figure 17. Monthly moisture free/ash free thiamine concentrations for the wholesale, coffin, and upright freezers.

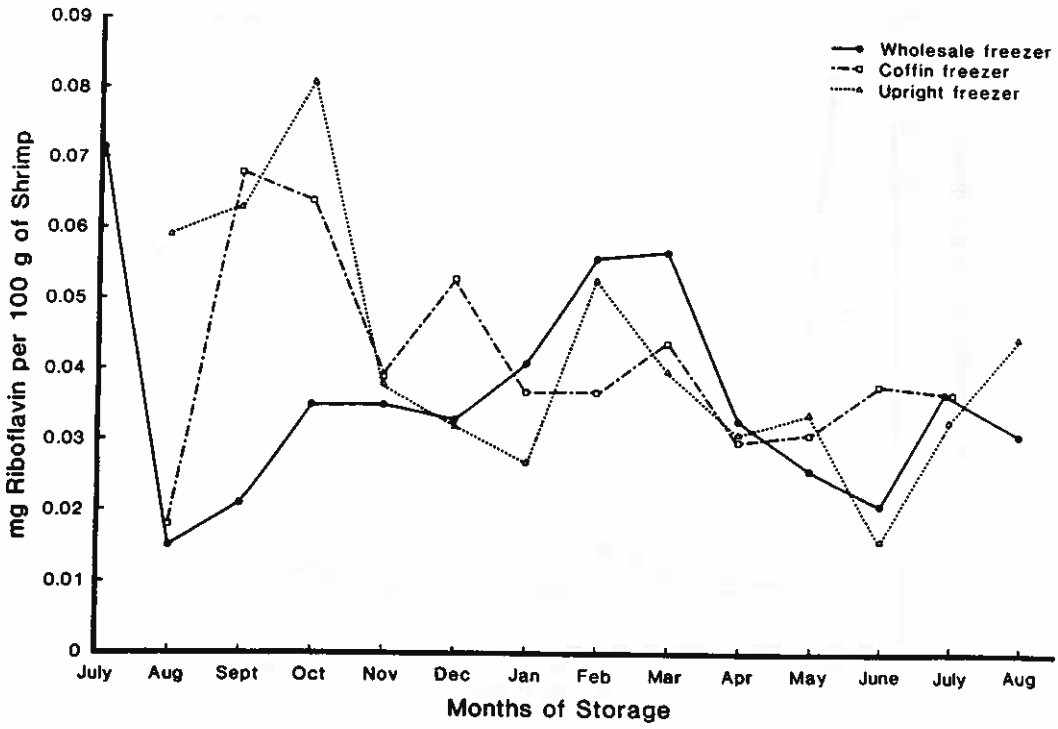

Figure 18. Mean monthly riboflavin levels for the warehouse, coffin, and upright freezers.

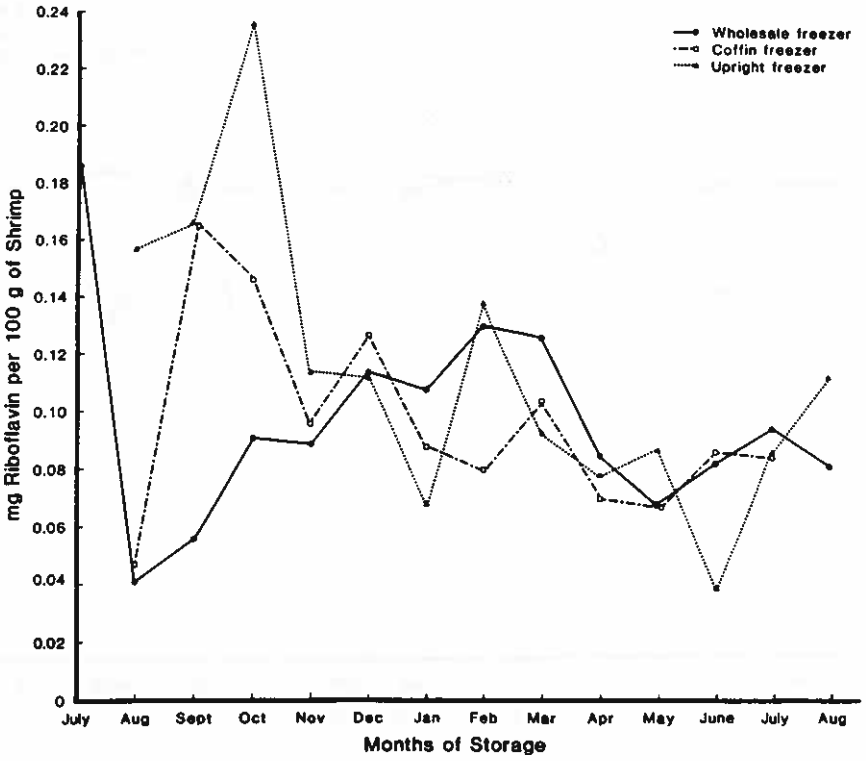

Figure 19. Mean monthly moisture free/ash free riboflavin levels for the warehouse, coffin and upright freezers.

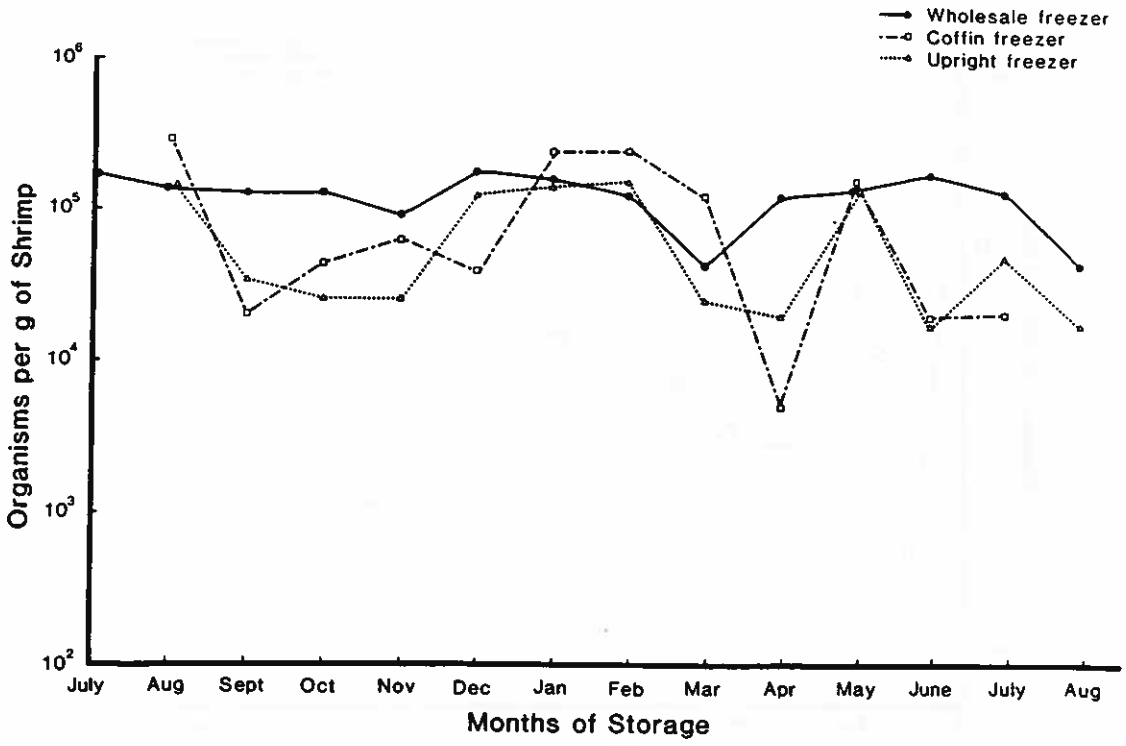

Figure 20. Mean monthly aerobic plate counts for the wholesale, coffin, and upright freezers.

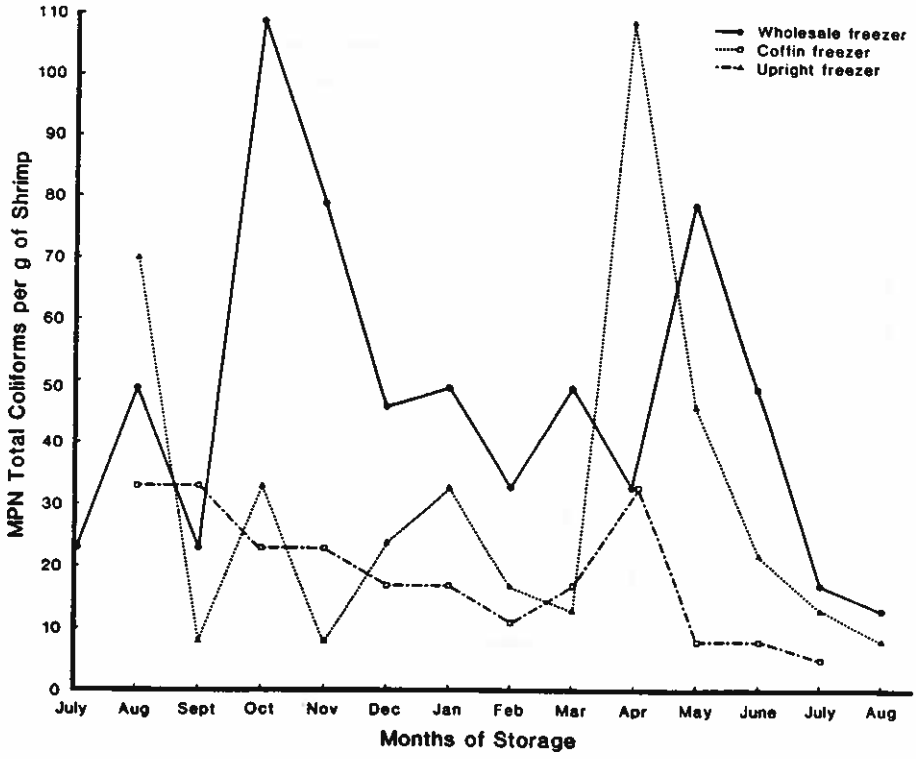

Figure 21. Monthly MPN total coliform organisms for the warehouse, coffin, and upright freezers.

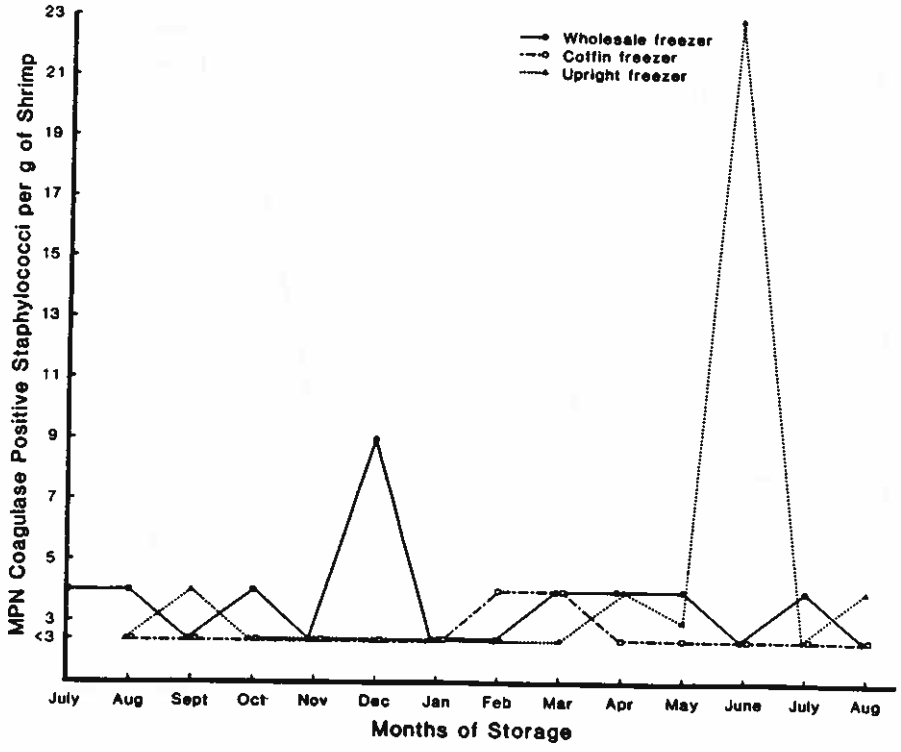

Figure 22. Monthly MPN coagulase positive staphylococci for the warehouse, coffin, and upright freezers.

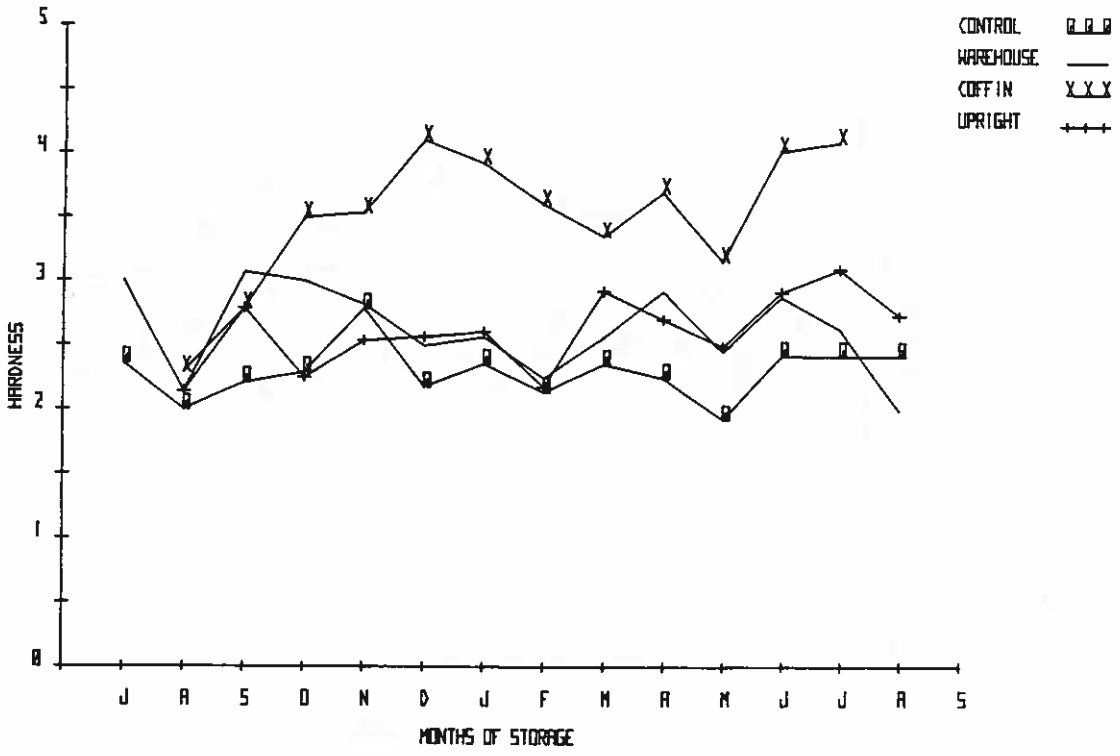

Figure 23. Mean monthly hardness levels for control, warehouse, coffin, and upright samples.

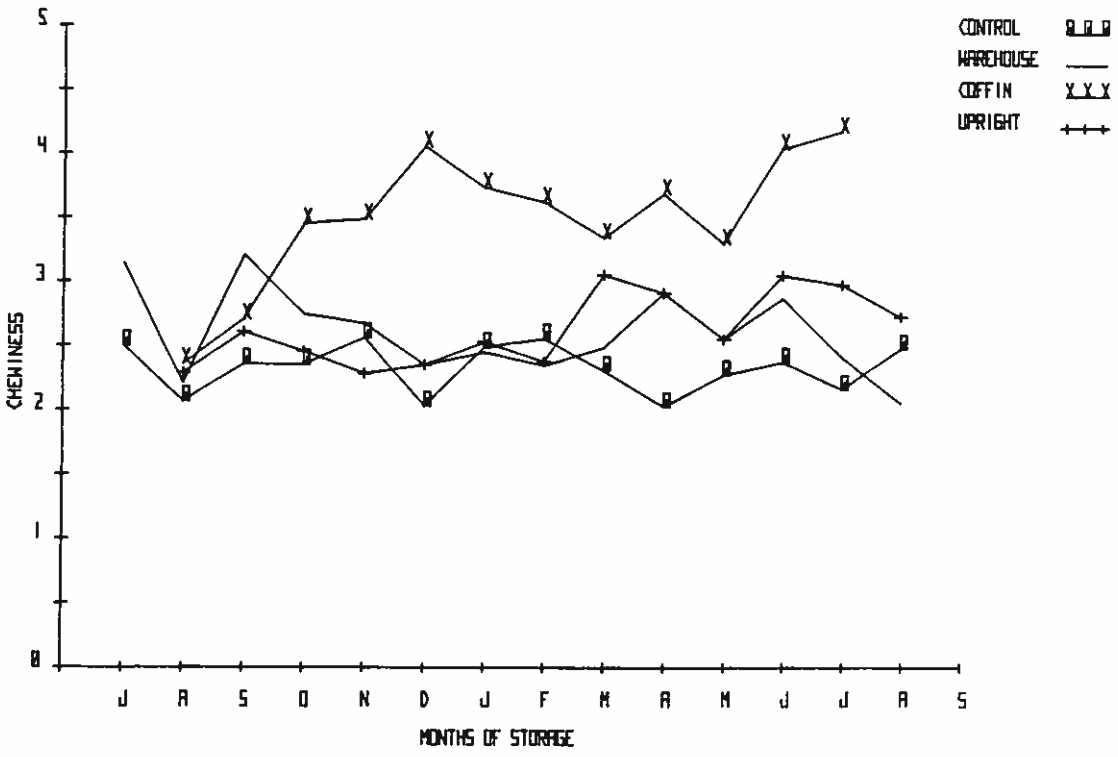

Figure 24. Mean monthly chewiness levels for control, warehouse, coffin, and upright samples.

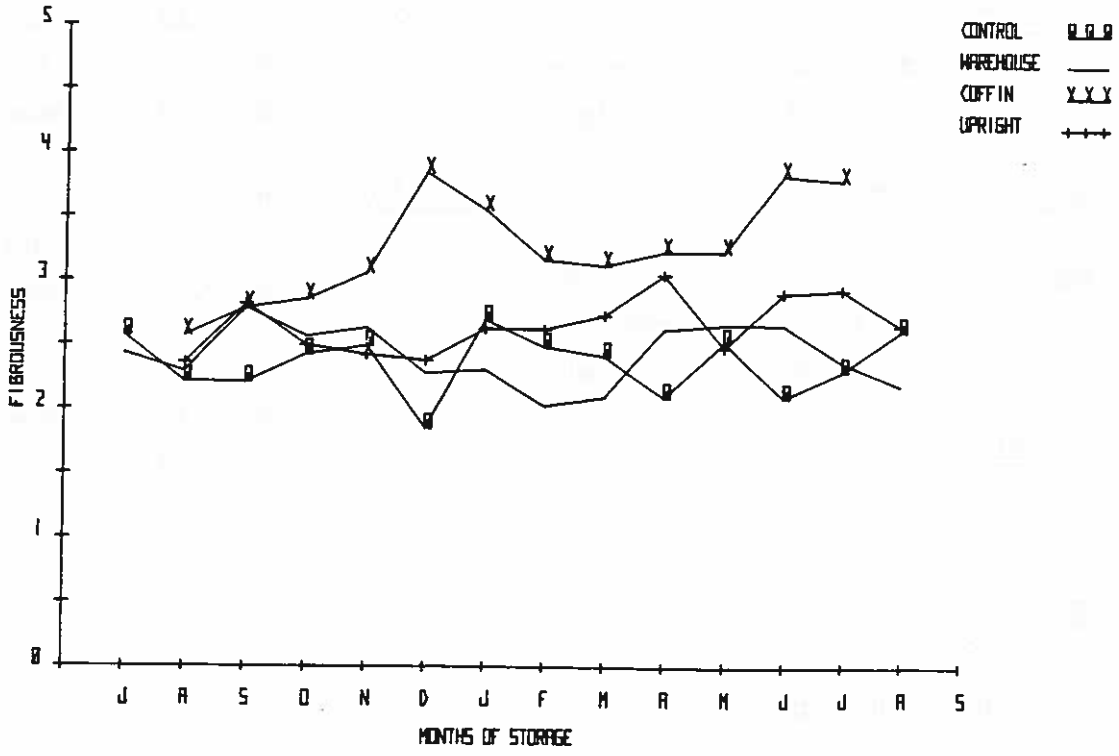

Figure 25. Mean monthly fibrousness levels for control, warehouse, coffin, and upright samples.

% PROTEIN

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		March 83	NSD	
Aug 82	NSD		April 83	NSD	
Sept 82	A 11.62	Coffin	May 83	A 13.10	Coffin
	BA 11.22	Warehouse		B 11.40	Upright
	B 10.12	Upright		B 11.09	Warehouse
Oct 82	NSD		June 83	A 13.52	Coffin
				B 10.84	Upright
Nov 82	A 12.60	Coffin		B 10.72	Warehouse
	BA 12.04	Warehouse			
	B 10.99	Upright	July 83	A 13.64	Coffin
				B 12.42	Warehouse
Dec 82	NSD			B 12.09	Upright
Jan 83	NSD		Aug 83	NSD	
Feb 83	NSD				

NSD = No significant difference

Table 17. Mean % protein levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

% ASH

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 3.30	Coffin
				B 2.94	Upright
Aug 82	NSD			B 2.89	Warehouse
Sept 82	A 3.11	Coffin	March 83	NSD	
	B 3.00	Warehouse			
	C 2.86	Upright	April 83	A 3.16	Coffin
				B 2.96	Warehouse
Oct 82	A 3.00	Warehouse		B 2.94	Upright
	BA 2.86	Coffin			
	B 2.56	Upright	May 83	A 3.36	Coffin
				BA 2.92	Upright
Nov 82	A 3.00	Coffin		B 2.84	Warehouse
	A 2.98	Warehouse			
	B 2.45	Upright	June 83	A 3.35	Coffin
				A 3.20	Upright
Dec 82	A 3.16	Coffin		B 2.82	Warehouse
	B 2.96	Upright			
	C 2.82	Warehouse	July 83	A 3.34	Coffin
				B 2.96	Warehouse
Jan 83	A 3.28	Coffin		C 2.88	Upright
	BA 3.13	Upright			
	B 2.93	Warehouse	Aug 83	A 3.25	Upright
				B 3.03	Warehouse

NSD = No significant difference

Table 18. Mean % ash significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

% MOISTURE FREE/ASH FREE PROTEIN

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 29.70	Upright
				A 29.12	Warehouse
Aug 82	NSD			B 25.34	Coffin
Sept 82	A 29.70	Warehouse	March 83	NSD	
	A 28.17	Coffin			
	B 26.18	Upright	April 83	NSD	
Oct 82	NSD		May 83	NSD	
Nov 82	NSD		June 83	NSD	
Dec 82	NSD		July 83	NSD	
Jan 83	NSD		Aug 83	NSD	

NSD = No significant difference

Table 19. Mean % moisture free/ash free protein levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

STANDARD PLATE COUNT
Org/g

Month	Mean	Freezer	Month	Mean	Freezer
July 82	----------		Feb 83	A 4.00×10^5	Coffin
				BA 1.85×10^5	Upright
Aug 82	A 4.50×10^5	Coffin		B 1.02×10^5	Warehouse
	B 1.41×10^5	Upright			
	B 1.24×10^5	Warehouse	March 83	A 1.24×10^5	Coffin
				B 6.30×10^4	Warehouse
Sept 82	A 1.03×10^5	Warehouse		B 4.15×10^4	Upright
	BA 5.25×10^4	Upright			
	B 2.95×10^4	Coffin	April 83	NSD	
Oct 82	NSD		May 83	NSD	
Nov 82	A 9.65×10^4	Warehouse	June 83	NSD	
	A 7.85×10^4	Coffin			
	B 4.20×10^4	Upright	July 83	A 1.28×10^5	Warehouse
				B 7.15×10^4	Upright
Dec 82	A 2.78×10^5	Warehouse		B 3.18×10^4	Coffin
	B 1.08×10^5	Upright			
	B 5.95×10^4	Coffin	Aug 83	A 6.60×10^4	Warehouse
				B 2.40×10^4	Upright
Jan 83	A 3.85×10^5	Coffin			
	B 2.22×10^5	Warehouse			
	B 1.70×10^5	Upright			

NSD = No significant difference

Table 20. Mean standard plate count, org/g, significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

HARDNESS

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 3.08	Warehouse	Feb 83	A 3.61	Coffin
	B 2.36	Control		B 2.25	Warehouse
				B 2.18	Upright
Aug 82	NSD			B 2.14	Control
Sept 82	A 3.08	Warehouse	March 83	A 3.36	Coffin
	A 2.79	Coffin		BA 2.93	Upright
	A 2.79	Upright		B 2.57	Warehouse
	B 2.21	Control		B 2.36	Control
Oct 82	A 3.50	Coffin	April 83	A 3.71	Coffin
	A 3.00	Warehouse		B 2.93	Warehouse
	B 2.29	Control		B 2.71	Control
	B 2.25	Upright		B 2.25	Upright
Nov 82	A 3.54	Coffin	May 83	A 3.17	Coffin
	B 2.82	Warehouse		B 2.50	Upright
	B 2.79	Control		B 2.46	Warehouse
	B 2.54	Upright		B 1.93	Control
Dec 82	A 4.11	Coffin	June 83	A 4.04	Coffin
	B 2.57	Upright		B 2.93	Upright
	B 2.50	Warehouse		B 2.89	Warehouse
	B 2.18	Control		B 2.43	Control
Jan 83	A 3.93	Coffin	July 83	A 4.07	Coffin
	B 2.61	Upright		B 3.11	Upright
	B 2.57	Warehouse		B 2.64	Warehouse
	B 2.36	Control		C 2.43	Control
			Aug 83	A 2.75	Upright
				BA 2.43	Control
				B 2.00	Warehouse

NSD = No significant difference

Table 21. Mean hardness significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

CHEWINESS

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 3.14	Warehouse	Feb 83	A 3.75	Coffin
	B 2.50	Control		B 2.57	Control
				B 2.39	Upright
Aug 82	NSD			B 2.36	Warehouse
Sept 82	A 3.21	Warehouse	March 83	A 3.36	Coffin
	BA 2.71	Coffin		BA 3.07	Upright
	BA 2.61	Upright		BC 2.50	Warehouse
	B 2.36	Control		C 2.32	Control
Oct 82	A 3.46	Coffin	April 83	A 3.71	Coffin
	B 2.75	Warehouse		B 2.93	Upright
	B 2.46	Upright		B 2.93	Warehouse
	B 2.36	Control		C 2.04	Control
Nov 82	A 3.50	Coffin	May 83	A 3.32	Coffin
	B 2.68	Warehouse		B 2.57	Upright
	B 2.58	Upright		B 2.57	Warehouse
	B 2.29	Control		B 2.29	Control
Dec 82	A 4.07	Coffin	June 83	A 4.07	Coffin
	B 2.36	Upright		B 3.07	Upright
	B 2.36	Warehouse		CB 2.89	Warehouse
	B 2.04	Control		C 2.39	Control
Jan 83	A 3.75	Coffin	July 83	A 4.21	Coffin
	B 2.54	Upright		B 3.00	Upright
	B 2.50	Control		C 2.43	Warehouse
	B 2.46	Warehouse		C 2.18	Control
			Aug 83	A 2.75	Upright
				BA 2.50	Control
				B 2.07	Warehouse

NSD = No significant difference

Table 22. Mean chewiness significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

increased from 2.57 - 3.82 and was significantly greater than other sample ratings from October 1982 through July 1983 (5 - 12 months of storage (Table 23).

Early product dessication was indicated by moistness ratings from the coffin freezer samples (Figure 26). Horizontal freezer moistness profiles were less than those perceived for the other sample conditions during the entire study. Coffin freezer values dropped from 2.93 - 1.14. Vertical freezer samples decreased from 3.29 - 2.75 and fell below the wholesale samples in May 1983. Wholesale moistness ratings increased from 2.79 - 2.93 by August 1983. The perceived moistness ratings of coffin freezer samples were significantly less than the warehouse or upright freezer samples for the third through the twelfth month of storage, except for March and May 1983 when no significant difference was detected (Table 24). Significantly greater moistness ratings than the other storage areas were given to horizontal samples in October and November 1982. The ratings correlated with significantly greater % moisture values determined for the same samples. Significant dessication in upright freezer samples was detected in the eleventh and twelfth months of storage when compared to the warehouse samples. The thirteenth month, one month after product was delivered to the vertical freezer from the wholesale freezer, showed no significant difference. Oily mouth coating values (Figure 27) failed to differentiate the three storage conditions (Table 25).

Textural properties of fried breaded shrimp perceived by a trained taste panel proved to be an effective method to detect quality changes in shrimp stored under wholesale and retail conditions. Panel members detected lower perceived moistness in samples from the coffin freezer as early as the first month of storage (significantly different by the third month). Decreased moistness was detected in the upright freezer samples by the tenth month of storage (significantly different by the eleventh month). Coffin freezer samples registered significantly greater hardness and chewiness ratings by the fourth month of storage while vertical freezer sample chewiness was differentiated by the eleventh month of storage. Textural changes in the wholesale shrimp was less than those observed in the retail samples. The greatest textural changes occurred in the coffin freezer samples and these changes were detected much earlier in the storage study than quality changes associated with the vertical freezer. Vertical freezer sample rating differences with storage time were greater than those perceived for the wholesale freezer.

Panel members readily detected flavor profile differences in breaded shrimp stored under the three experimental conditions. The detected level of overall shrimp flavor decreased from 1.65 - 0.71 for the coffin freezer and 1.78 - 1.71 for the vertical freezer (Figure 28). Overall shrimp flavor detected from coffin freezer shrimp was significantly less than the other samples by the fifth month of the study while the vertical

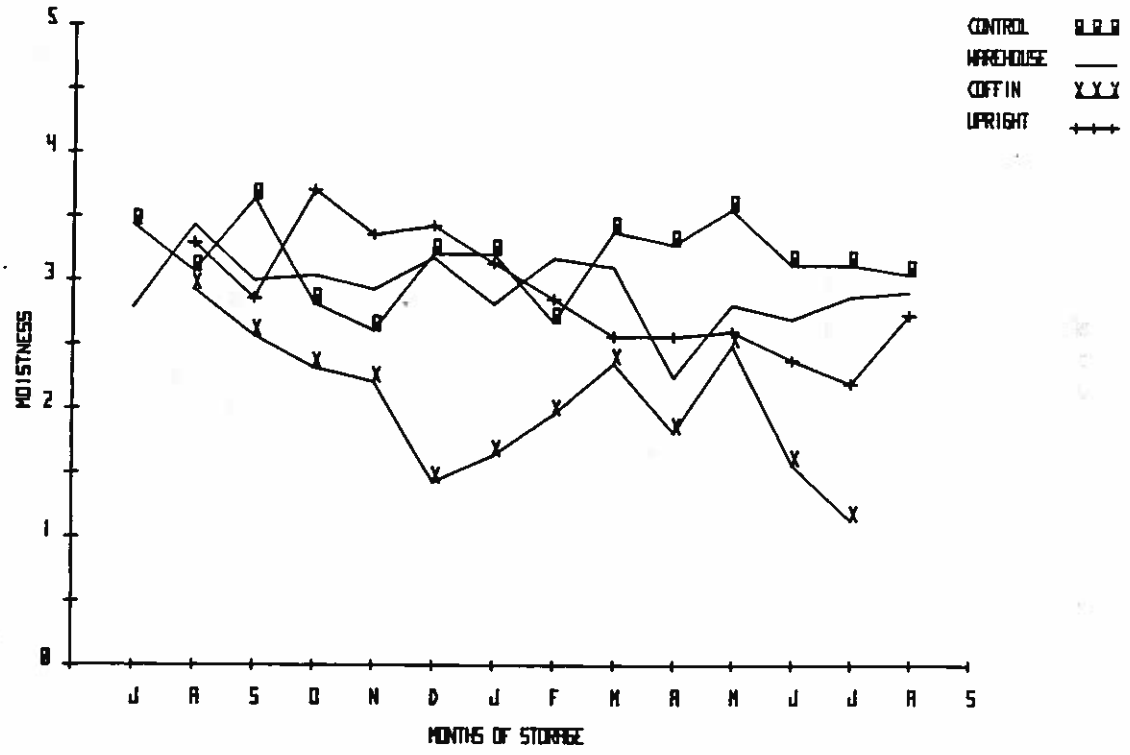

Figure 26. Mean monthly moistness levels for control, warehouse, coffin, and upright samples.

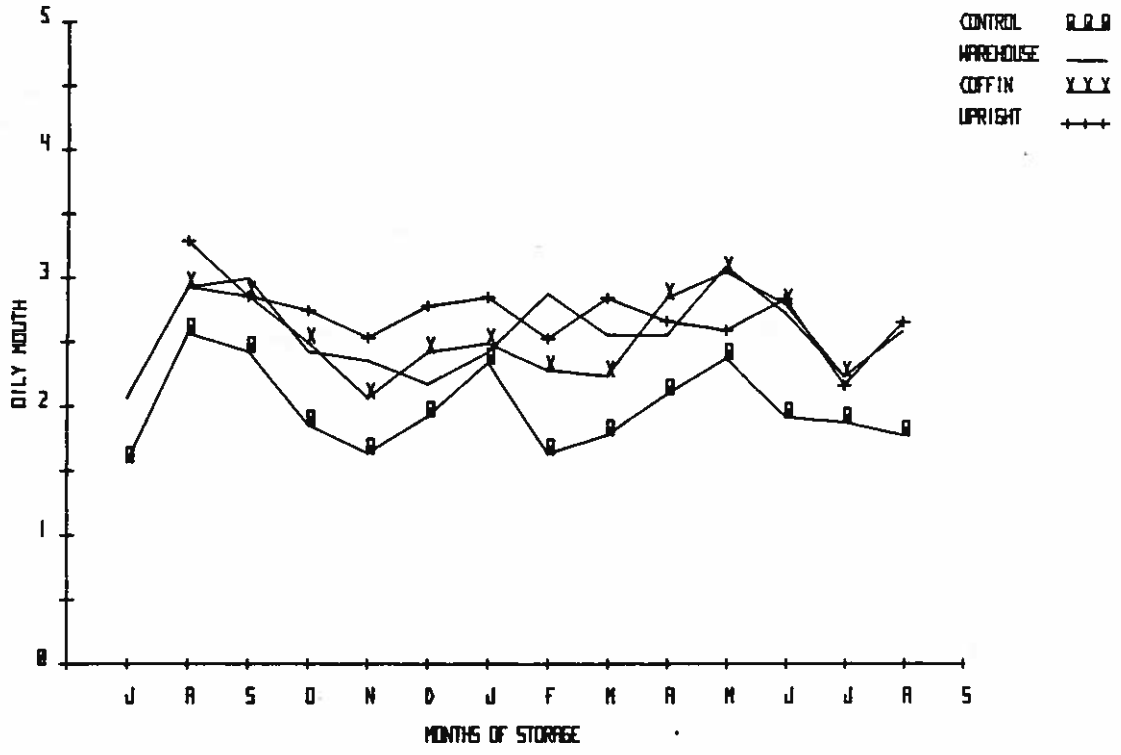

Figure 27. Mean monthly oily mouth coating levels for control, warehouse, coffin, and upright samples.

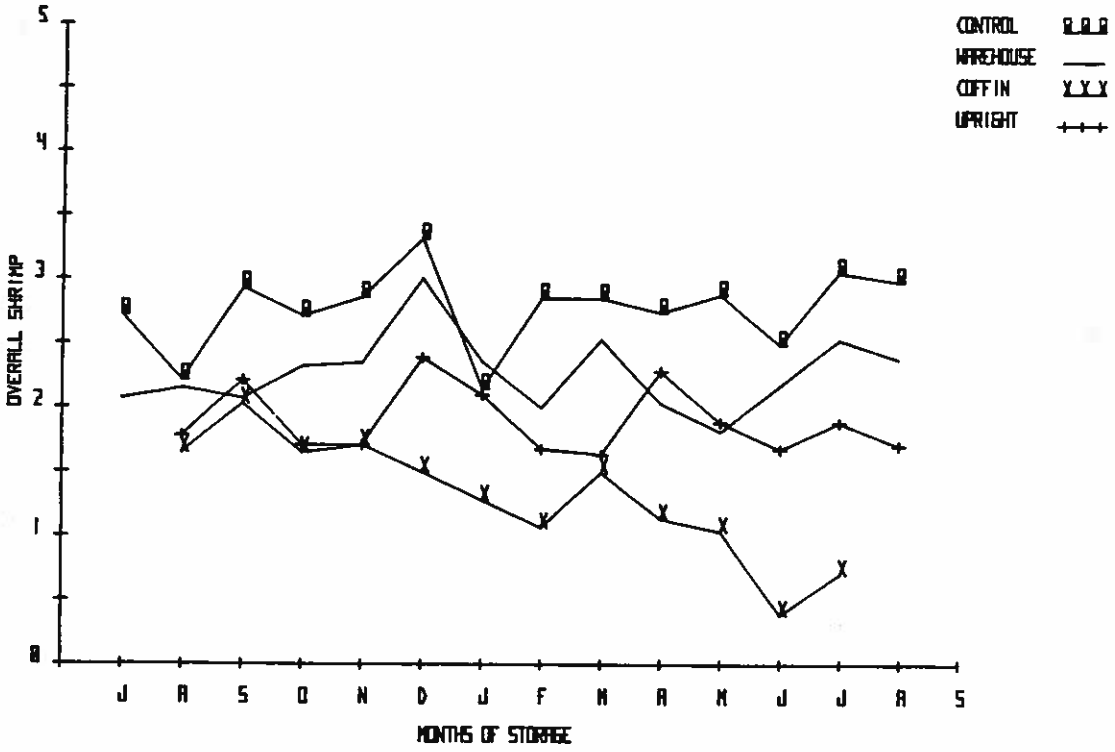

Figure 28. Mean monthly overall shrimp intensity for control, warehouse, coffin, and upright samples.

FIBROUSNESS

Month	Mean	Freezer	Month	Mean	Freezer
July 82	NSD		March 83	A 3.14	Coffin
				BA 2.75	Upright
Aug 82	NSD			B 2.43	Control
				B 2.11	Warehouse
Sept 82	A 2.82	Upright			
	A 2.79	Coffin	April 83	A 3.25	Coffin
	A 2.79	Warehouse		A 3.07	Upright
	B 2.21	Control		BA 2.64	Warehouse
				B 2.11	Control
Oct 82	NSD				
			May 83	A 3.25	Coffin
Nov 82	NSD			BA 2.68	Warehouse
				B 2.54	Control
Dec 82	A 3.86	Coffin		B 2.50	Upright
	B 2.39	Upright			
	B 2.29	Warehouse	June 83	A 3.86	Coffin
	C 1.86	Control		B 2.93	Upright
				CB 2.68	Warehouse
Jan 83	A 3.57	Coffin		C 2.11	Control
	B 2.71	Control			
	B 2.64	Upright	July 83	A 3.82	Coffin
	B 2.32	Warehouse		B 2.96	Upright
				B 2.39	Warehouse
Feb 83	A 3.18	Coffin		B 2.32	Control
	B 2.57	Upright			
	B 2.50	Control	Aug 83	NSD	
	B 2.04	Warehouse			

NSD = No significant difference

Table 23. Mean fibrousness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

MOISTNESS

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 3.43	Control	Feb 83	A 3.18	Warehouse
	B 2.79	Warehouse		A 2.86	Upright
				A 2.68	Control
Aug 82	NSD			B 1.96	Coffin
Sept 82	A 3.64	Control	March 83	A 3.39	Control
	BA 3.00	Warehouse		BA 3.11	Warehouse
	B 2.86	Upright		B 2.57	Upright
	B 2.57	Coffin		B 2.36	Coffin
Oct 82	A 3.71	Upright	April 83	A 3.29	Control
	B 3.04	Warehouse		B 2.57	Upright
	CB 2.82	Control		CB 2.25	Warehouse
	C 2.32	Coffin		C 1.82	Coffin
Nov 82	A 3.71	Upright	May 83	A 3.57	Control
	B 3.04	Warehouse		B 2.82	Warehouse
	CB 2.82	Control		B 2.61	Upright
	C 2.32	Coffin		B 2.50	Coffin
Dec 82	A 3.43	Upright	June 83	A 3.14	Control
	A 3.21	Control		BA 2.71	Warehouse
	A 3.18	Warehouse		B 2.39	Upright
	B 1.43	Coffin		C 1.57	Coffin
Jan 83	A 3.21	Control	July 83	A 3.14	Control
	A 3.14	Upright		A 2.89	Warehouse
	A 2.82	Warehouse		B 2.21	Upright
	B 1.64	Coffin		C 1.14	Coffin
			Aug 83	NSD	

NSD = No significant difference

Table 24. Mean moistness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

OILY MOUTH COATING

Month	Mean	Freezer	Month	Mean	Freezer
July 82	NSD		March 83	A 2.86	Upright
				A 2.61	Coffin
Aug 82	A 3.29	Upright		A 2.57	Warehouse
	BA 2.93	Coffin		B 1.79	Control
	BA 2.93	Warehouse			
	B 2.57	Control	April 83	A 2.86	Coffin
				BA 2.68	Upright
Sept 82	NSD			BA 2.57	Warehouse
				B 2.11	Control
Oct 82	A 2.75	Upright			
	BA 2.50	Coffin	May 83	A 3.11	Warehouse
	BA 2.43	Warehouse		A 3.07	Coffin
	B 1.86	Control		BA 2.61	Upright
				B 2.39	Control
Nov 82	A 2.46	Upright			
	A 2.36	Warehouse	June 83	A 2.86	Upright
	BA 2.07	Coffin		A 2.82	Coffin
	B 1.64	Control		A 2.75	Warehouse
				B 1.93	Control
Dec 82	NSD		July 83	NSD	
Jan 83	NSD		Aug 83	A 2.68	Upright
				A 2.61	Warehouse
Feb 83	A 2.89	Warehouse		B 1.79	Control
	BA 2.54	Upright			
	B 2.29	Coffin			
	C 1.64	Control			

NSD = No significant difference

Table 25. Mean oily mouth coating levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

freezer shrimp levels remained significantly less than the wholesale shrimp following the eleventh month of storage (Table 26). Nutty buttery ratings (Figure 29) exhibited the same pattern as the overall shrimp values except for May 1983 which showed no significant differences between the experimental samples (Table 27). New product had been transferred from the warehouse freezer to both retail freezers in April. Sweet levels (Figure 30) showed significantly lower levels for the coffin freezer samples for the eleventh and twelfth months of storage (Table 28). Detected old seafood flavors (Figure 31) increased most rapidly in the coffin stored shrimp (1.64 - 3.39) and significantly exceeded the levels of the other samples by the third month of storage (Table 29). The levels remained greater than the other areas except for the November 1982 and April 1983 samples which were collected one month after restocking the coffin freezer. Perceived old seafood flavors decreased for the wholesale (1.29 - 0.29) and vertical retail (2.07 - 1.68) freezers with time, but the wholesale level fell more rapidly and remained significantly below the vertical freezer ratings by the eleventh month of the study (Table 29). Additionally, the old seafood flavor ratings for October and November 1983 showed the vertical freezer samples to be significantly greater than the warehouse shrimp. The data reflect temperature abuse detected in the upright freezer at that time (Figure 3), when maximum defrost temperatures exceeded 0°C. The February 1983 upright freezer sample had a significantly higher old seafood rating than the warehouse freezer. The February sample was collected three months after the last product was transferred to the upright freezer. Detectable freezer burn (Figure 32) increased with time for both the coffin freezer samples (1.14 - 4.14) and the vertical freezer samples (1.86 - 2.54). Wholesale freezer burn ratings decreased from 0.93 - 0.75. Freezer burn results closely parallelled the patterns developed by overall shrimp flavor, nutty buttery flavor, and old seafood flavor. The coffin freezer had significantly greater ratings, indicating poorer quality than the upright and warehouse freezers from the third through the twelfth month of storage (Table 30). The temperature abuse detected in the upright freezer registered as significantly greater freezer burn than the warehouse samples in October and November 1982. February and March 1983 rated significantly higher freezer burn levels than the warehouse freezer, 3 and 4 months, respectively, after the freezer was restocked. The final three months of the storage study provided significantly greater freezer burn levels for vertical freezer samples than warehouse samples. Rancidity ratings (Figure 33) increased with storage time for the coffin freezer samples (1.07 - 2.04) while the other samples decreased with time. The coffin freezer rancidity levels were significantly greater than warehouse values from the third through twelfth months (Table 31). Again the wholesale freezer levels (0.70 - 0) fell more rapidly than the vertical freezer samples (1.43 - 0.36).

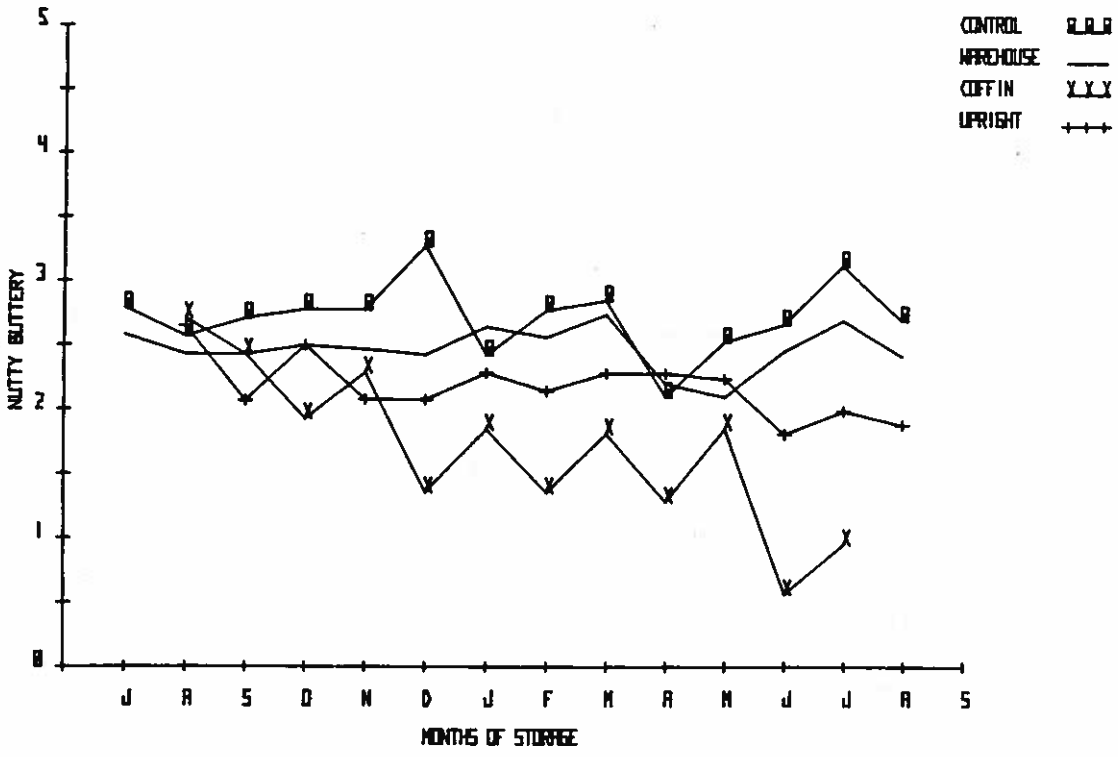

Figure 29. Mean monthly nutty buttery flavor for control, warehouse, coffin, and upright samples.

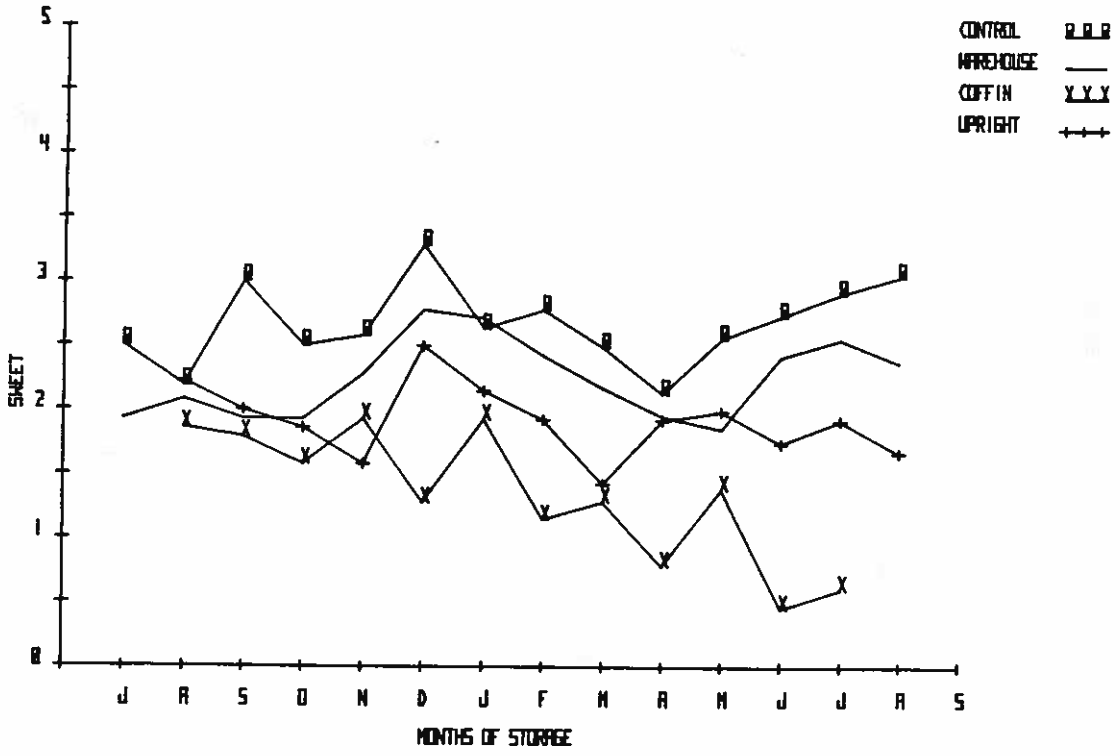

Figure 30. Mean monthly sweet intensity for control, warehouse, coffin, and upright samples.

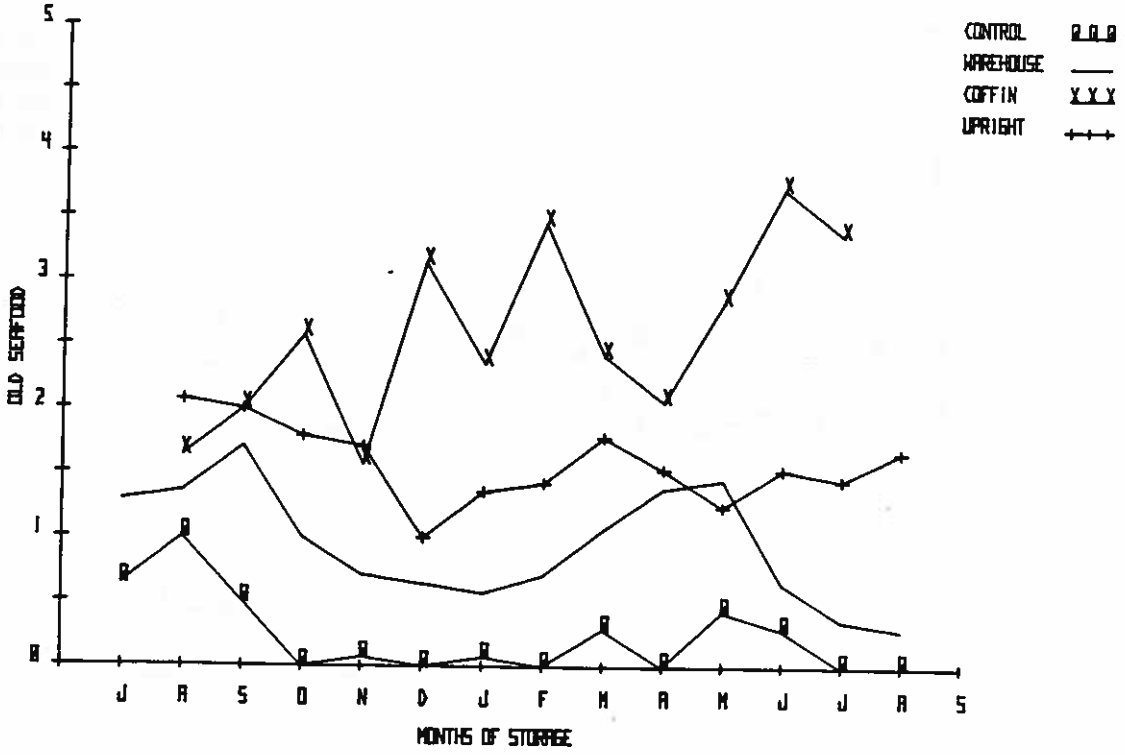

Figure 31. Mean monthly old seafood flavor for control, warehouse, coffin, and upright samples.

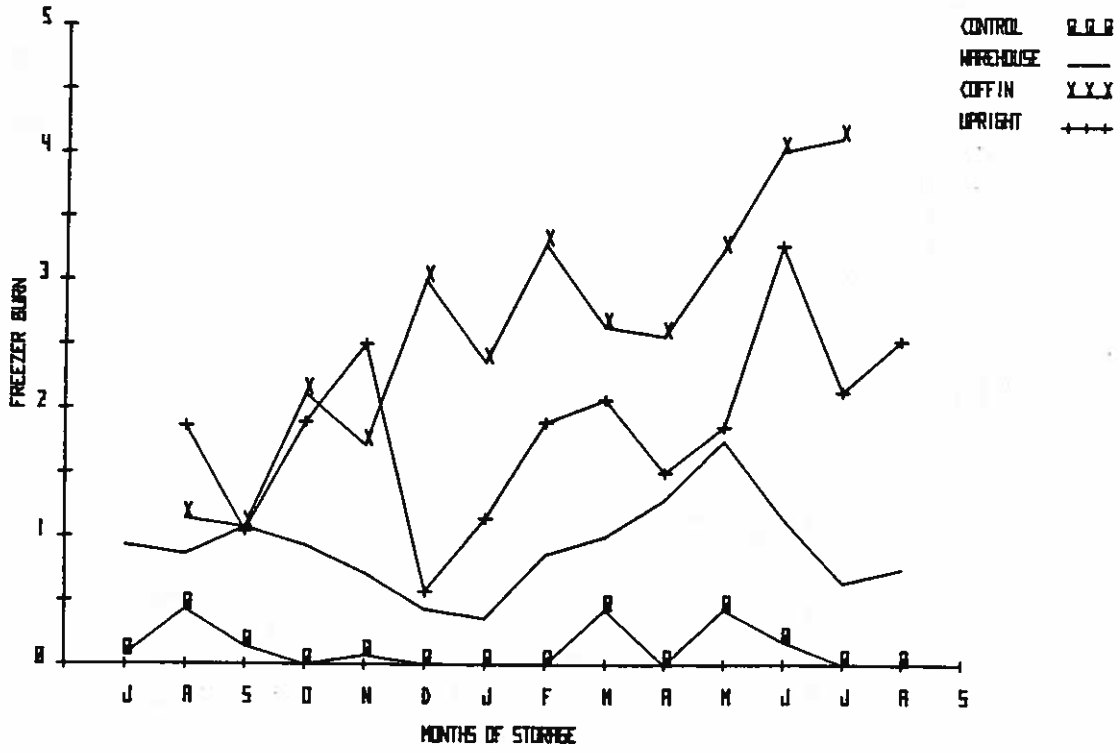

Figure 32. Mean monthly freezer burn flavor for control, warehouse, coffin, and upright samples.

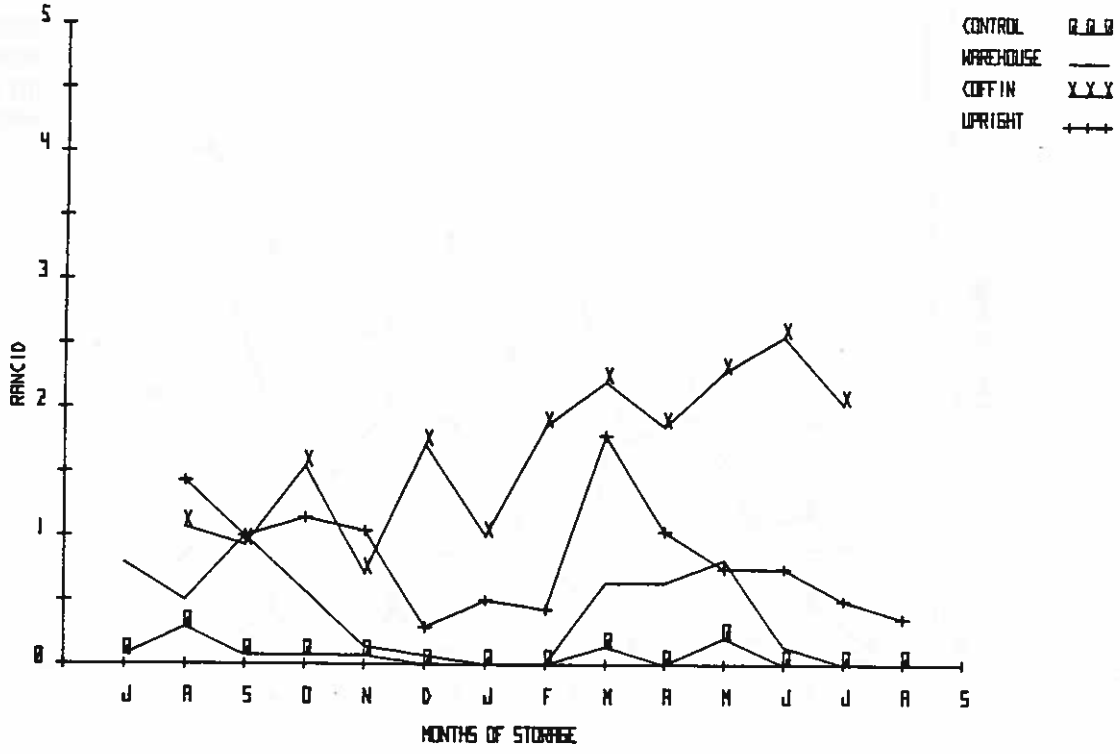

Figure 33. Mean monthly rancid flavor for control, warehouse, coffin, and upright samples.

OVERALL SHRIMP

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 2.71	Control	Feb 83	A 2.86	Control
	B 2.07	Warehouse		B 2.00	Warehouse
				B 1.68	Upright
Aug 82	A 2.21	Control		C 1.07	Coffin
	A 2.14	Warehouse			
	BA 1.79	Upright	March 83	A 2.86	Control
	B 1.64	Coffin		A 2.54	Warehouse
				B 1.64	Upright
Sept 82	A 2.93	Control		B 1.50	Coffin
	B 2.21	Upright			
	B 2.07	Warehouse	April 83	A 2.75	Control
	B 2.04	Coffin		BA 2.29	Upright
				B 2.04	Warehouse
Oct 82	A 2.79	Control		C 1.14	Coffin
	BA 2.32	Warehouse			
	BC 1.71	Upright	May 83	A 2.89	Control
	C 1.64	Coffin		B 1.89	Upright
				B 1.82	Warehouse
Nov 82	A 2.86	Control		C 1.04	Coffin
	A 2.36	Warehouse			
	B 1.71	Coffin	June 83	A 2.50	Control
	B 1.71	Upright		A 2.25	Warehouse
				B 1.75	Upright
Dec 82	A 3.32	Control		C 0.39	Coffin
	A 3.00	Warehouse			
	B 3.39	Upright	July 83	A 3.07	Control
	C 1.50	Coffin		A 2.53	Warehouse
				B 1.89	Upright
Jan 83	A 2.36	Warehouse		C 0.71	Coffin
	A 2.14	Control			
	A 2.11	Upright	Aug 83	A 3.00	Control
	B 1.29	Coffin		B 2.39	Warehouse
				C 1.71	Upright

Table 26. Mean overall shrimp levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

NUTTY BUTTERY

Month	Mean	Freezer
July 82	NSD	
Aug 82	NSD	
Sept 82	A 2.71	Control
	BA 2.43	Coffin
	BA 2.43	Warehouse
	B 2.07	Upright
Oct 82	A 2.79	Control
	BA 2.50	Upright
	BA 2.50	Warehouse
	B 1.93	Coffin
Nov 82	NSD	
Dec 82	A 3.29	Control
	B 2.43	Warehouse
	B 2.29	Upright
	C 1.36	Coffin
Jan 83	A 2.64	Warehouse
	BA 2.43	Control
	BA 2.29	Upright
	B 1.86	Coffin
Feb 83	A 2.79	Control
	BA 2.57	Warehouse
	B 2.14	Upright
	C 1.36	Coffin
March 83	A 2.86	Control
	A 2.75	Warehouse
	BA 2.29	Upright
	B 1.82	Coffin
April 83	A 2.29	Upright
	A 2.14	Warehouse
	A 2.11	Control
	B 1.29	Coffin
May 83	A 2.93	Control
	B 2.25	Upright
	B 2.11	Warehouse
	B 1.86	Coffin
June 83	A 2.68	Control
	A 2.46	Warehouse
	B 1.82	Upright
	C 0.57	Coffin
July 83	A 3.14	Control
	A 2.71	Warehouse
	B 2.04	Upright
	C 0.96	Coffin
Aug 83	A 2.71	Control
	A 2.43	Warehouse
	B 1.89	Coffin

NSD = No significant difference

Table 27. Mean nutty buttery levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

SWEET

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 2.50	Control	Feb 83	A 2.79	Control
	B 1.93	Warehouse		A 2.43	Warehouse
				B 1.93	Upright
Aug 82	NSD			C 1.14	Coffin
Sept 82	A 3.00	Control	March 83	A 2.50	Control
	B 2.00	Upright		A 2.18	Warehouse
	B 1.93	Warehouse		B 1.43	Upright
	B 1.79	Coffin		B 1.29	Coffin
Oct 82	A 2.50	Control	April 83	A 2.14	Control
	BA 1.93	Warehouse		A 1.96	Warehouse
	BA 1.86	Upright		A 1.93	Upright
	B 1.57	Coffin		B 0.79	Coffin
Nov 82	A 2.57	Control	May 83	A 2.57	Control
	BA 2.29	Warehouse		BA 2.00	Upright
	BC 1.93	Coffin		BA 1.93	Warehouse
	C 1.57	Upright		B 1.39	Coffin
Dec 82	A 3.29	Control	June 83	A 2.75	Control
	BA 2.79	Warehouse		A 2.43	Warehouse
	B 2.50	Upright		B 1.75	Upright
	C 1.29	Coffin		C 0.46	Coffin
Jan 83	A 2.71	Warehouse	July 83	A 2.93	Control
	BA 2.64	Control		BA 2.57	Warehouse
	BA 2.14	Upright		B 1.93	Upright
	B 1.93	Coffin		C 0.61	Coffin
			Aug 83	A 3.07	Control
				A 2.39	Warehouse
				B 1.68	Upright

NSD = No significant difference

Table 28. Mean sweetness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

OLD SEAFOOD

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 1.36	Warehouse	Feb 83	A 3.46	Coffin
	B 0.64	Control		B 1.43	Upright
				C 0.71	Warehouse
Aug 82	A 2.07	Upright		D 0.00	Control
	BA 1.64	Coffin			
	BA 1.36	Warehouse	March 83	A 2.43	Coffin
	B 1.00	Control		BA 1.79	Upright
				B 1.07	Warehouse
Sept 82	A 2.00	Coffin		C 0.29	Control
	A 2.00	Upright			
	A 1.71	Warehouse	April 83	A 2.07	Coffin
	B 0.50	Control		A 1.54	Upright
				A 1.39	Warehouse
Oct 82	A 2.57	Coffin		B 0.00	Control
	B 1.79	Upright			
	C 1.00	Warehouse	May 83	A 2.86	Coffin
	D 0.00	Control		B 1.46	Warehouse
				CB 1.25	Upright
Nov 82	A 1.79	Upright		C 0.43	Control
	A 1.57	Coffin			
	B 0.71	Warehouse	June 83	A 3.75	Coffin
	C 0.07	Control		B 1.54	Upright
				C 0.64	Warehouse
Dec 82	A 3.14	Coffin		C 0.29	Control
	B 1.00	Upright			
	B 0.64	Warehouse	July 83	A 3.39	Coffin
	C 0.07	Control		B 1.46	Upright
				C 0.36	Warehouse
Jan 83	A 2.36	Coffin		C 0.00	Control
	B 1.36	Upright			
	CB 0.64	Warehouse	Aug 83	A 1.68	Upright
	C 0.14	Control		B 0.29	Warehouse
				B 0.00	Control

NSD = No significant difference

Table 29. Mean old seafood levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

FREEZER BURN

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 0.93	Warehouse	Feb 83	A 3.29	Coffin
	B 0.07	Control		B 1.89	Upright
				C 0.86	Warehouse
Aug 82	A 1.86	Upright		D 0.00	Control
	BA 1.14	Coffin			
	B 0.86	Warehouse	March 83	A 2.64	Coffin
	B 0.43	Control		A 2.07	Upright
				B 1.00	Warehouse
Sept 82	A 1.07	Coffin		B 0.43	Control
	A 1.07	Warehouse			
	A 1.04	Upright	April 83	A 2.57	Coffin
	B 0.14	Control		B 1.50	Upright
				B 1.29	Warehouse
Oct 82	A 2.11	Coffin		C 0.00	Control
	A 1.89	Upright			
	B 0.93	Warehouse	May 83	A 3.25	Coffin
	C 0.00	Control		B 1.86	Upright
				B 1.75	Warehouse
Nov 82	A 2.29	Upright		C 0.05	Control
	A 1.71	Coffin			
	B 0.71	Warehouse	June 83	A 4.04	Coffin
	B 0.07	Control		B 2.29	Upright
				C 1.14	Warehouse
Dec 82	A 3.00	Coffin		D 0.18	Control
	B 0.57	Upright			
	CB 0.43	Warehouse	July 83	A 4.14	Coffin
	C 0.00	Control		B 2.14	Upright
				C 0.64	Warehouse
Jan 83	A 2.36	Coffin		C 0.00	Control
	B 1.14	Upright			
	CB 0.36	Warehouse	Aug 83	A 2.54	Upright
	C 0.00	Control		B 0.75	Warehouse
				C 0.00	Control

NSD = No significant difference

Table 30. Mean freezer burn levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

RANCID

Month	Mean	Freezer	Month	Mean	Freezer
July 82	A 0.79	Warehouse	Feb 83	A 1.86	Coffin
	B 0.71	Control		B 0.43	Upright
				B 0.00	Control
Aug 82	A 1.43	Upright		B 0.00	Warehouse
	BA 1.07	Coffin			
	BC 0.50	Warehouse	March 83	A 2.21	Coffin
	C 0.36	Control		A 1.79	Upright
				B 0.64	Warehouse
Sept 82	A 1.00	Upright		B 0.14	Control
	A 1.00	Warehouse			
	A 0.93	Coffin	April 83	A 1.86	Coffin
	B 0.07	Control		B 1.04	Upright
				CB 0.64	Warehouse
Oct 82	A 1.54	Coffin		C 0.00	Control
	BA 1.14	Upright			
	BC 0.57	Warehouse	May 83	A 2.29	Coffin
	C 0.07	Control		B 0.82	Warehouse
				B 0.75	Upright
Nov 82	A 1.18	Upright		B 0.21	Control
	A 0.71	Coffin			
	B 0.14	Warehouse	June 83	A 2.57	Coffin
	B 0.07	Control		B 0.75	Upright
				B 0.14	Warehouse
Dec 82	A 1.71	Coffin		B 0.00	Control
	B 0.29	Upright			
	B 0.07	Warehouse	July 83	A 2.04	Coffin
	B 0.00	Control		B 0.50	Upright
				B 0.00	Control
Jan 83	A 1.00	Coffin		B 0.00	Warehouse
	BA 0.50	Upright			
	B 0.00	Control	Aug 83	A 0.36	Upright
	B 0.00	Warehouse		B 0.00	Control
				B 0.00	Warehouse

NSD = No significant difference

Table 31. Mean rancidity levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

Modified flavor profiles of fried frozen breaded shrimp effectively differentiated samples collected from the three storage conditions. Sample deterioration was detected within three to five months of storage for the coffin freezer samples, and within 11 months of storage for the vertical freezer samples. Samples stored in the wholesale freezer exhibited the least changes during storage.

CONCLUSIONS AND RECOMMENDATIONS

The nutritional quality of frozen breaded shrimp showed little deterioration following 13 months of storage at the wholesale and retail level. Protein, thiamine, and riboflavin levels revealed no differences between a warehouse freezer, an upright freezer, and a horizontal coffin freezer. Vitamin levels decreased rapidly following the first two months of storage; however, levels remained faily constant for the next 11 months and within 0.007 mg/100g of published U.S. Department of Agriculture nutritional data (13).

Monthly monitoring of percent moisture, net weights, ammonium levels, and organoleptic qualities proved effective methods to evaluate product quality and differentiate frozen breaded shrimp stored in a wholesale warehouse freezer, a retail upright freezer, and a retail horizontal freezer. Product stored in the wholesale freezer exhibited the least deterioration with time. The quality of the retail product decreased much more rapidly in the coffin freezer, showing more rapid and earlier deterioration than the upright freezer. Approximately four months into the study, the quality of shrimp stored in the retail coffin freezer was visibly less than that associated with the vertical retail freezer or the warehouse freezer. Product stored in the horizontal freezer was exposed to consistently greater temperature abuse than was encountered in the vertical or warehouse freezer. Daily minimum temperatures were consistently 5 - 10°C greater than the minimum upright freezer temperatures, while little difference in daily maximum temperatures was noted. Freezer temperatures exceeded 0°C in 7 months of the study. Variations between maximum and minimum temperatures for the retail freezers was approximately 12°C for the coffin freezer and 14 - 18°C for the upright freezer. The defrost cycles were 6 - 8 hours and 6 - 12 hours, respectively. The vertical freezer exceeded 0°C in 4 months of the study. Temperature abuse in October and November 1982 was readily detected by chemical and organoleptic monitoring of product stored in the upright freezer. The warehouse freezer exhibited the lowest mean temperatures with only a 2 - 3°C daily temperature variation. Organoleptic evaluation against the control product indicated no consistent significant differences following 13 months of storage. Eleven months into the study, noticeable and consistent quality deterioration was detected in the vertical freezer samples. Product samples from the vertical freezer in February and March 1983 (3 and 4 months after breaded shrimp had been restocked) showed significantly different organoleptic qualities than those observed

for the wholesale freezer. Product quality in the coffin freezer was judged to be below minimum consumer standards by the end of the study.

The results of the study indicate that significant organoleptic deterioration can be detected as early as three to four months after production when shrimp are stored in a retail freezer. Net weight and % moisture changes support the contention. As the age of the product increases, shorter storage times at the retail level will precipitate measureable organoleptic and chemical deterioration within the product. Storage at the wholesale level below -20°C proved to be an effective method to maintain the quality of frozen breaded shrimp for at least 13 months of storage.

All tested product failed to meet the U.S. Standard of Identity for frozen breaded shrimp, of a minimum 50% shrimp content, despite the fact that the breaded product contained 51.5% shrimp before freezing. Product held in the warehouse freezer had the greatest shrimp levels after 13 months of storage. Williams (14) and Rao (10) suggest rapid freezing and the use of finer grained breading to reduce the relative loss of percent shrimp through moisture migration. The introduction of moisture barrier packaging would help alleviate quality and regulatory problems associated with moisture migration.

An educational program should be developed to apprise retail merchants of the importance of temperature control and good product handling technqiues in the maintenance of proper quality for all frozen foods, but especially seafoods. The current study indicated that the greatest quality deterioration occurred at the retail level, however, consumer dissatisfaction and regulatory action is generally directed to the original manufacturer. Additional handling abuses are probable while the product is being transported from wholesale to retail locations. The effects of product transportation on quality were not addressed by this study. Development of improved product packaging, breading formulations, and the training of retail personnel would greatly increase the quality of frozen breaded shrimp offered to the consuming public.

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THE EFFECT OF SALINITY ON FLAVOR CHARACTERISTICS OF PENAEID SHRIMP

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INTRODUCTION

Aquaculture is rapidly becoming an important source of fish and shellfish on a worldwide basis. However, the ultimate success of an aquaculture program depends on commercial feasibility and consumer acceptance of the cultured species. For a product to be produced in an economically competitive manner, it must be in demand by the consumer. Consumer acceptance is based on characteristics such as texture, color, aroma and flavor. If these characteristics can be manipulated during culture to suit consumer preferences, a high demand for the product can be achieved, resulting in an economically feasible and therefore successful aquaculture program.

Free amino acids in penaeid shrimp tissue increase with increasing salinity as a result of their role in osmoregulation (3, 5). Since free amino acids have been found to be important contributors to flavor in marine products (4, 6), flavor manipulation to suit consumer preferences could be possible through changes in environmental salinity. The objectives of this research were to determine the effect of environmental salinity on moisture content, salt content, free amino acid concentration and organoleptic characteristics of penaeid shrimp tissue.

METHODS AND MATERIALS

Moisture, salt and free amino acid levels as a function of environmental salinity.

Brown shrimp, *Penaeus aztecus*, caught in the Intercoastal Waterway were obtained from bait stands in the vicinity of Corpus Christi, Texas. In the first trial, 25 shrimp were stocked into each of six 50-gallon aquaria at 30 parts per thousand (ppt) salinity and 28°C. Mean length and weight were 9.97 cm and 8.49 g, respectively. Following a 72-hour acclimation period, one shrimp was removed from each tank to obtain initial values for moisture, salt and free amino acids (FAA). The salinity in two tanks remained at 30 ppt while the salinities in the remaining four were then changed to 10 and 50 ppt, with two tanks at each salinity. Salinity changes were made at a rate of 2 ppt/hour, with a maximum of 10 ppt/day, using appropriate amounts of either freshwater or a concentrated salt solution. Once final salinities were reached, the water in each tank was periodically exchanged to maintain water quality and to prevent excessive ammonia levels in the tanks. Shrimp samples, two shrimp per tank, were taken 24 hours after the final salinities were reached and at 48 hour intervals thereafter for a period of nine days.

In the second trial, 28 shrimp were stocked into each of six 50-gallon aquaria maintained at 25 ppt salinity and 25.5°C. Mean length and weight of the shrimp were 8.91 cm and 6.09 g, respectively. Two shrimp were removed from each tank following a 24-hour acclimation period to obtain initial values for moisture, salt and free amino acids. Salinities were then changed to 10 ppt in three tanks, and 50 ppt in the remaining three tanks. Salinity changes were made simultaneously using appropriate amounts of freshwater or concentrated salt solution. As in the first trial, periodic exchanges of the water in each tank was necessary to maintain water quality. Shrimp samples were taken at 1, 2, 4, 8, 12, 24 and 48 hours after final salinities were reached.

For both phases of the experiment, each shrimp was deheaded, peeled and deveined immediately upon removal from the aquaria. The shrimp were analyzed for moisture, salt and free amino acids (reported as amino acid nitrogen, (AAN)).

Moisture content was determined according to AOAC procedure (1). Two-gram samples of shrimp were dried in an air oven for 16-18 hours at 100-102°C. Samples were removed from the oven, cooled in a desiccator and weighed.

Salt content was measured according to AOAC procedure (1) using the Volumetric Method. Total chloride is reported as sodium chloride.

Amino acid nitrogen was determined using a modification of the copper procedure of Spies and Chambers (7) as described by Cobb et al. (2). Shrimp extracts were prepared by blending shrimp in a Waring blender at a ratio of 1 g shrimp to 2 ml of 7% trichloroacetic acid (TCA) solution. To 4 ml of shrimp extract, 6 ml of cupric sulfate suspension was added. The solution was mixed carefully and allowed to stand for 20 minutes. This mixture was then centrifuged, and the supernatant removed and read against a reagent blank at 630 nm using a Beckman spectrophotometer.

Sensory evaluation of shrimp grown at different salinities

Sample preparation - *Penaeus vannamei* were obtained from the Texas A&M Shrimp Mariculture Project facility at Flour Bluff. Mean length and weight were 12.8 cm and 16.11 g, respectively. Shrimp were stocked into nine 1,000 gallon tanks at the Texas A&M Shrimp Mariculture facility at Port Aransas. Initial mean tank salinity was 25 ppt. Fifty-five to 44 shrimp were stocked into each of the tanks. Following a 24-hour acclimation period, salinities were changed to either 10, 30 or 50 ppt, with three tanks at each salinity. Rate of salinity change was a maximum of 7 ppt/ppt using appropriate amounts of freshwater or rock salt, depending on the desired salinity.

The shrimp remained at the new salinities for 72 hours and were then harvested, deheaded and held on ice for 24 hours. Samples were taken from each salinity for determination of moisture and salt content, AA-N, and amino acid profiles. All shrimp were then glazed and stored at 25°C until needed.

For sensory evaluation, the shrimp were thawed under cold running water, boiled in distilled water for 3 minutes and placed into an ice bath for rapid cooling to prevent further cooking. Once cooled, the shrimp were peeled and held at 5°C for presentation to a trained taste panel.

Panel Selection and Training - Potential panelists were screened with a series of triangle tests before being selected for the taste panel. Screening sessions were held twice a day for 4 days, with two triangle tests presented at each session. Only the top ten candidates were selected for the final panel. Those who were selected were trained in daily sessions until consistent judgements were obtained from each panelists. Individual performance records were kept to help monitor progress and identify problem areas.

Sensory Evaluation - Two different taste tests were conducted: a triangle test and a flavor analysis. The triangle test was used to determine the presence or absence of flavor differences, as well as the degree of difference between shrimp from the various salinities. Flavor analysis was used to evaluate flavor characteristics of shrimp from 10 and 50 ppt salinity.

Testing was done in the Meat Science sensory testing rooms at Texas A&M University. Panelists were assigned individual booths to keep distractions to a minimum and to prevent communication among panelists. Testing booths were connected to the food preparation area by a hooded dome through which the samples were passed. The booths were set with a napkin, pencil, appropriate questionaire (Figures 1 and 2), rinse cup (distilled water at ambient temperature) and expectorant cup. The shrimp were coded with three-digit random numbers and served on white styrofoam plates.

Three comparisons were made in the triangle test: 10 ppt vs 30 ppt; 10 ppt vs 50 ppt; and 30 ppt vs 50 ppt. Panelists were presented three shrimp, one odd and two duplicate samples, and asked to identify the odd sample and assess the degree of difference between the samples (Figure 1).

The level of significance used was 5%. With ten panelists testing each comparison three times, 16 correct responses were required (8).

Flavor analysis, utilizing an unstructured scale (Figure 2), was used to evaluate flavor characteristics. The scale is a 6-inch horizontal line with anchor points 0.5 inches from each end. The panelists were asked to make a vertical line across the horizontal line to indicate the magnitude of the characteristics in question. Numerical values were assigned to each rating by measuring the distance of a panelist's mark from the left of the line in units of 0.1 inches. Values for each flavor characteristics were tabulated and an analysis of variance was conducted to detect significant differences between the samples.

RESULTS AND DISCUSSION

Gradual Salinity Change

Moisture - Figure 3 shows the effect of salinity on tissue moisture through time. Moisture content appeared to increase (decrease) with decreasing (increasing) salinity. Analysis of variance showed the effect of salinity on moisture content to be significant. Results of Duncan's Multiple Range test using pooled variances showed significant differences in moisture content between shrimp from 10 and 50 ppt until day 7. Differences in moisture content between the 10 and 30 ppt shrimp were significant only on days 3 and 5. Significant differences were found between the 30 and 50 ppt shrimp until day 5.

Changes in moisture content were more pronounced for salinity increases than for salinity decreases. It appears that adjustment of moisture content is the immediate response to changes in environmental osmotic pressure. Moisture content seems to gradually return to normal once free amino acids are present at high enough concentrations to balance external osmotic pressure (Fig. 6).

Internal salt - Total salt content is reported on both a wet and dry weight basis to compensate for fluctuating moisture content. Figures 4 and 5 show the temporal response of tissues to gradual changes in environmental salinity. Analysis of variance (α=0.05) showed a significant effect of salinity on salt content (wet and dry weight). According to a Duncan's test using pooled variances, salt content differed significantly between 10 and 50 ppt shrimp and 30 and 50 ppt shrimp. Although salt content of the 10 ppt shrimp was usually lower than that of the 30 ppt shrimp, the differences were not significant.

Amino Acid Nitrogen - As with salt content, AA-N is also reported on a wet and dry weight basis. The effect of salinity on free amino acids is shown in Figures 6 and 7. Free amino acids significantly (α=0.05) increase with increasing salinity and decrease with decreasing salinity. The same effect was reported by Cobb et al. (3) using *P. stylirostris*, and by McCoid (5) using *P. vannamei* and *P. setiferus*.

Analysis of variance showed significant differences in AA-N on both wet and dry weight basis. Results of a Duncan's test using pooled variances showed the 50 ppt shrimp to be significantly different from both the 10 and 30 ppt shrimp. No significant differences were observed between the 10 and 30 ppt shrimp; therefore, changes in AA-N are more pronounced in response to salinity increases than to salinity decreases.

Simultaneous Salinity Change

Moisture - Figure 8 shows the effect of salinity on moisture content in response to a very rapid change in salinity. As observed in the gradual salinity change, moisture content significantly decreased with increasing salinity and increased with decreasing salinity; however, the adjustment in moisture content was made more rapidly. A dramatic decrease in moisture content of the 50 ppt shrimp occurred within the first 2 hours. This rapid decrease can perhaps be explained as an immediate attempt to achieve an equilibrium between internal and environmental osmotic concentrations. Moisture content continued to decrease until 12 hours then very gradually increased. Increasing moisture is concurrent with increasing AA-N, indicating that FAA are assuming a primary role as the osmoregulatory agent. Moisture content in the 10 ppt shrimp increased slightly then returned to normal after 12 hours. Analysis of variances showed a significant effect of salinity on moisture content (α=0.05).

Internal salt - The effect of environmental salinity on total internal salt concentration is shown in Figures 9 (wet weight basis) and 10 (dry weight basis). Salt content increased with increasing salinity and decreased with decreasing salinity. Adjustments in internal salt concentration made to salinity increases were apparently more pronounced than those made to salinity decreases. Maximum salt content of the 50 ppt

shrimp was at 8 hours while minimum salt content of the 10 ppt shrimp was at 4 hours. Analysis of variance (α=0.05) of both wet and dry weight values showed the effect of salinity on internal salt concentration to be significant.

Amino Acid Nitrogen - Figures 11 and 12 show the effect of salinity on free amino acid concentration following a simultaneous salinity change. As seen in results from the gradual salinity change, FAA increased with increasing salinity and decreased with decreasing salinity; however, adjustments in FAA levels were apparently made more rapidly following the simultaneous salinity change than were made following the gradual change. Adjustments to salinity increases were more pronounced than adjustments to salinity decreases.

Analysis of variance (α=0.05) of FAA on a wet weight basis showed significant differences between the 10 and 50 ppt shrimp. However, analysis of FAA on the dry weight basis showed significant differences existed only after 12 hours. Differences between FAA levels on a wet and dry weight basis probably represent fluctuations in moisture content.

Sensory Evaluation - Table 1 shows the results of the triangle tests. A significant difference (α=0.05) in flavor was shown between 10 and 50 ppt shrimp, and between 30 and 50 ppt shrimp; however differences in flavor were only slight. No significant difference was found between 10 and 30 ppt shrimp. This is probably due to a non-linear relationship between FAA's and salinity (5).

Results of the flavor analysis are shown in Table 2. The shrimp from 50 ppt salinity were significantly (α=0.05) shrimpier, sweeter, nuttier and saltier than the shrimp from 10 ppt. No significant difference was found in the intensity of the chemical-metallic characteristics.

CONCLUSIONS

Environmental salinity has an inverse effect on moisture content and a direct effect on internal salt and free amino acid concentration of penaeid shrimp tissue. Responses to salinity increase were more pronounced than to salinity decreases. The immediate response to salinity changes was an adjustment in moisture content followed closely by a change in internal salt concentration. Once FAA's were present at sufficient levels to balance external osmotic pressure (day 3 and 12 hours for gradual and simultaneous changes, respectively) moisture content gradually returned to pre-change levels. From this data, it appears that shrimp respond to changes in osmotic pressure in two ways: moisture gains or losses, and changes in free amino acid concentration. Internal salt appeared to play only a minor role in osmoregulation, especially at lower salinities.

Flavor manipulation to suit consumer preferences is possible in penaeid shrimp. Since FAA's increase with increasing salinity, and FAA's are the primary flavor producers in marine products (4, 6), shrimp from higher salinities (>30 ppt) are more flavorful.

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TABLE 1. Results of Triangle Test

COMPARISON	# CORRECT RESPONSES	% CORRECT RESPONSES
10 vs 50	18	60
10 vs 30	13	43
30 vs 50	18	60

TABLE 2. Mean intensities of the various flavor characteristics

FLAVOR CHARACTERISTIC	TREATMENT	
	10 ppt	50 ppt
SHRIMPY	2.61	3.25
SWEET	1.81	3.23
CHEMICAL-METALLIC	0.95	0.80
NUTTY	1.28	1.98
SALTY	1.17	1.50

TRIANGLE TEST

NAME _____ DATE _____

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample.

code	Check the odd sample
	_____
	_____
	_____

2. Indicate the degree of difference between the duplicate samples and the odd sample.

slight _____

moderate _____

much _____

extreme _____

3. Comments:

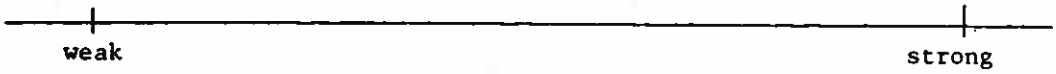
Figure 1 - Questionaire used for a triangle test

FLAVOR ANALYSIS

NAME _____ DATE _____

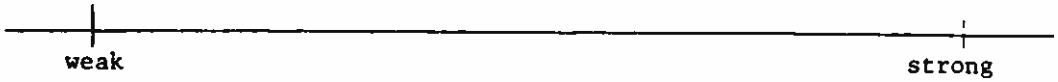
Please make a vertical line on the horizontal line to indicate the intensity of the specified characteristic of each sample.

1. shrimpy



2. sweet

3. chemical-metallic



4. nutty

5. salty

6. Comments:

Figure 2. Questionaire used for flavor analysis

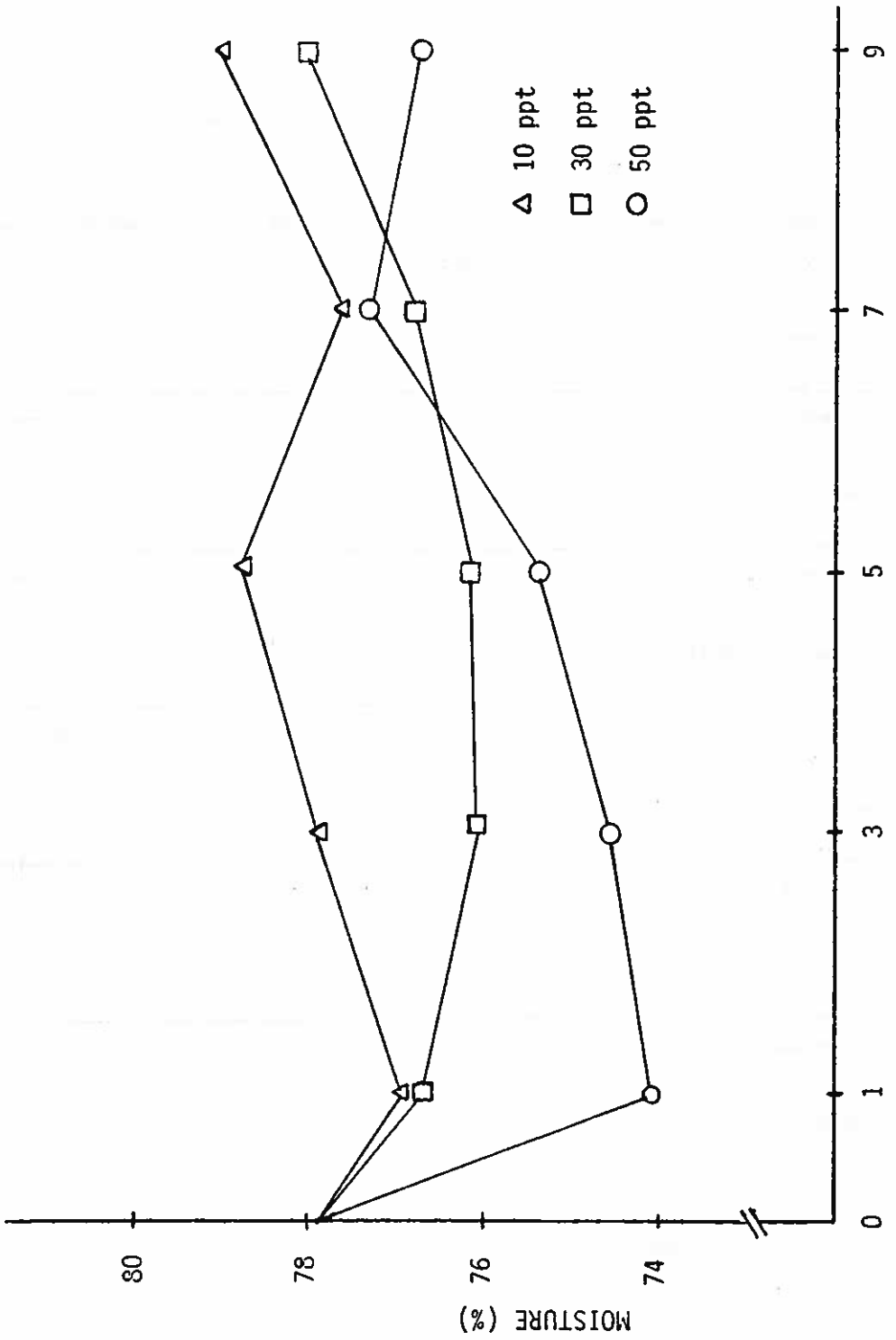


Figure 3. Moisture content following gradual salinity changes

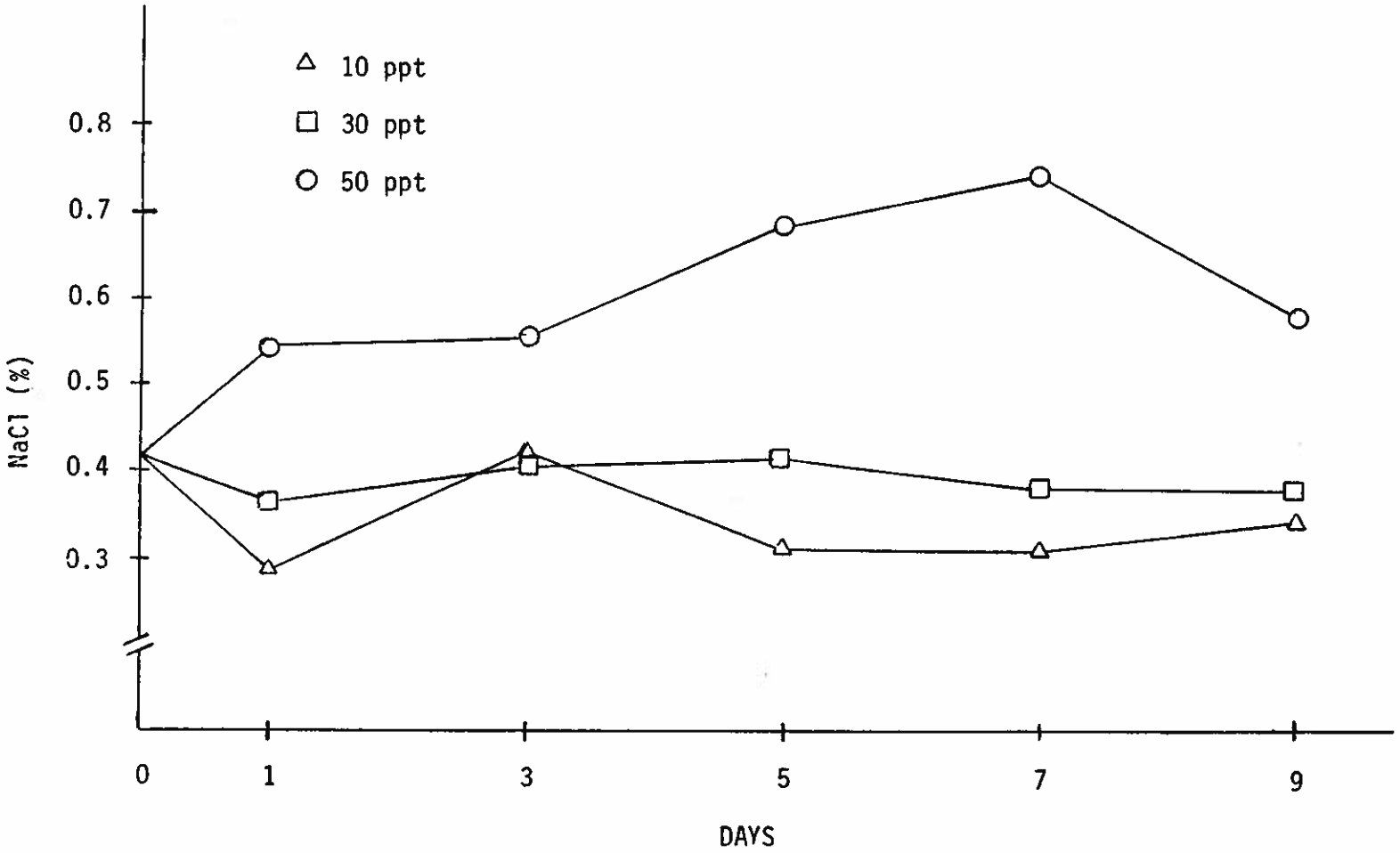


Figure 4. Sodium chloride on a wet weight basis following gradual salinity change

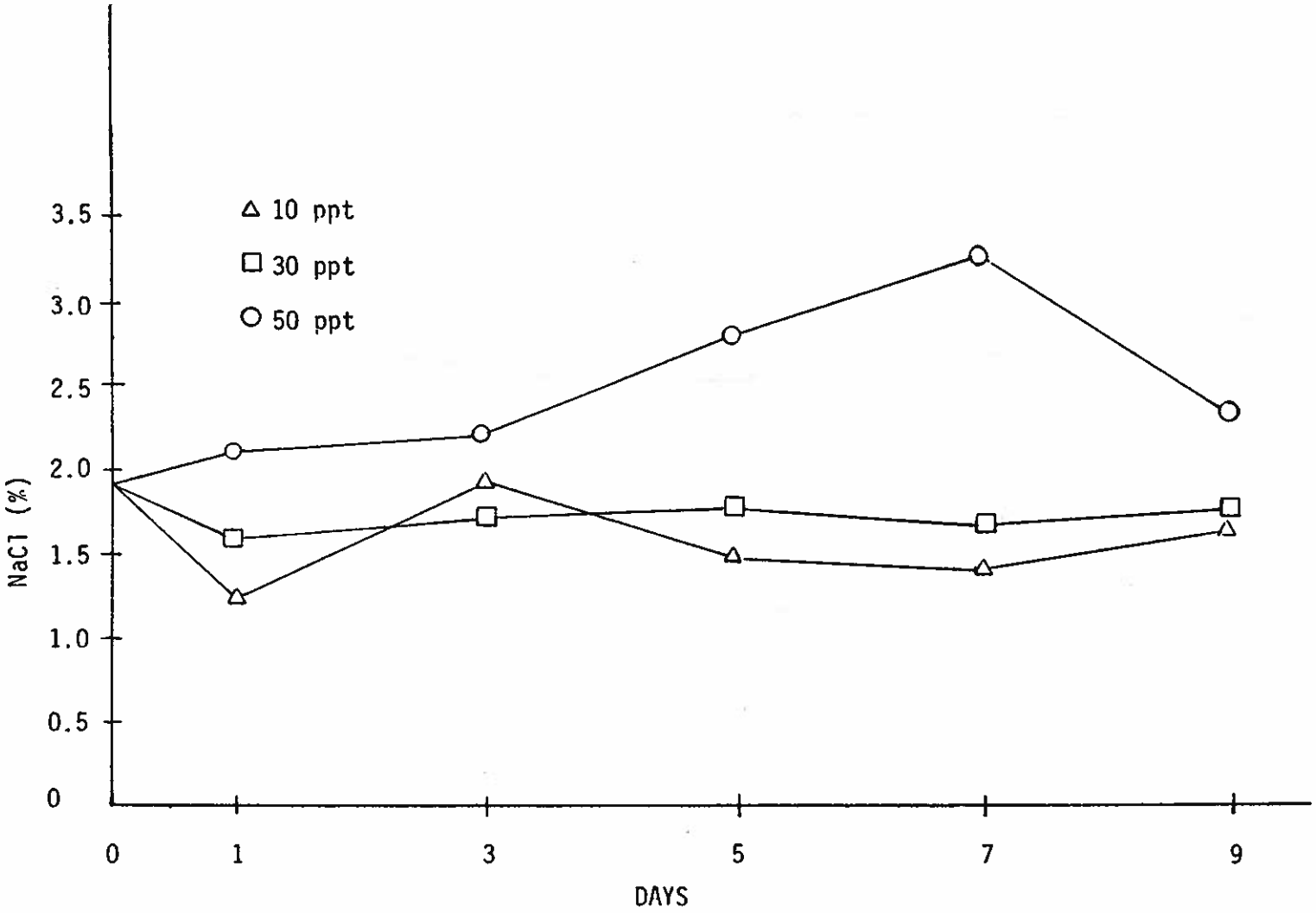


Figure 5. Sodium chloride following a gradual salinity change - dry weight basis

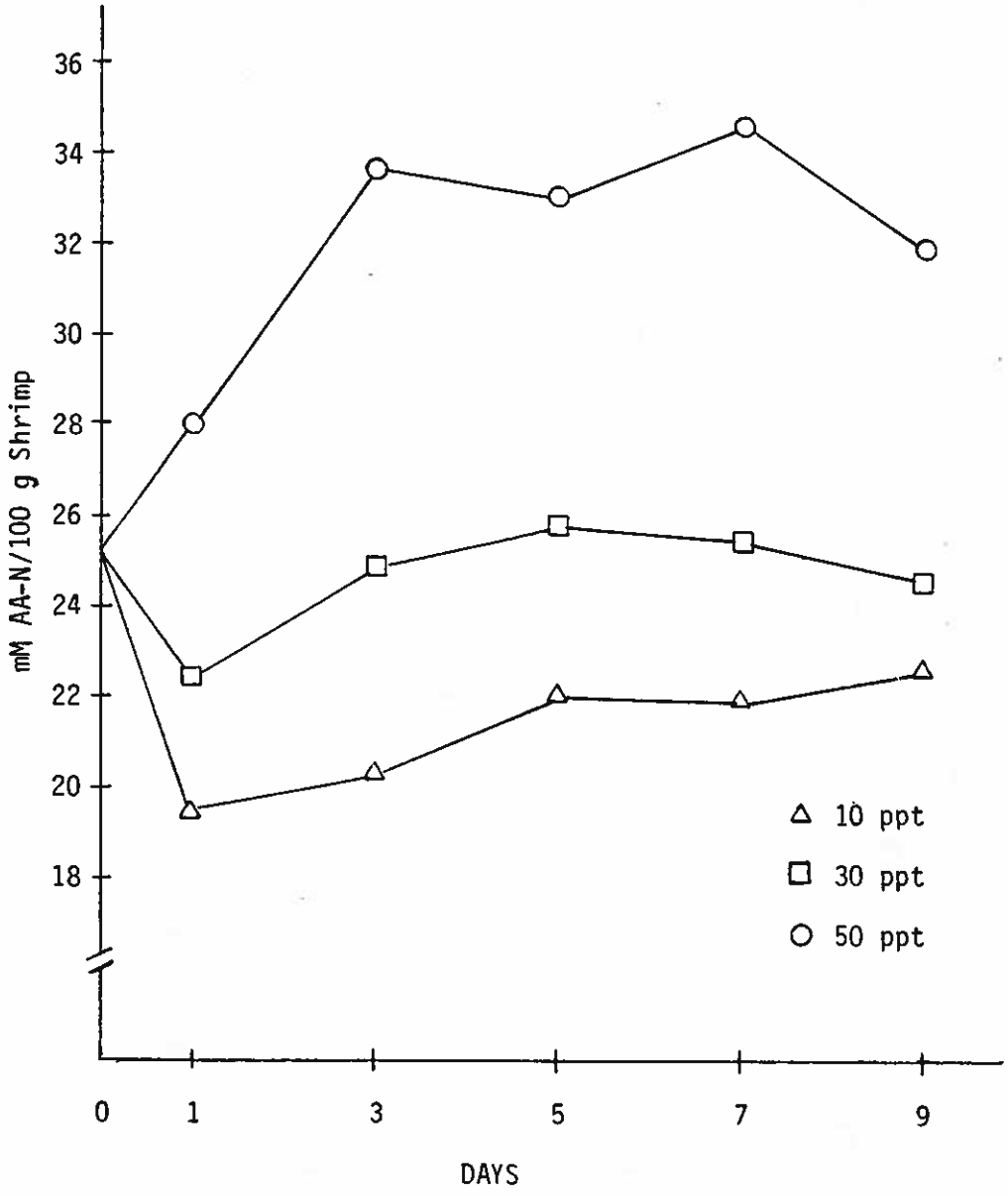


Figure 6. Amino Acid nitrogen on a wet weight basis following a gradual salinity change

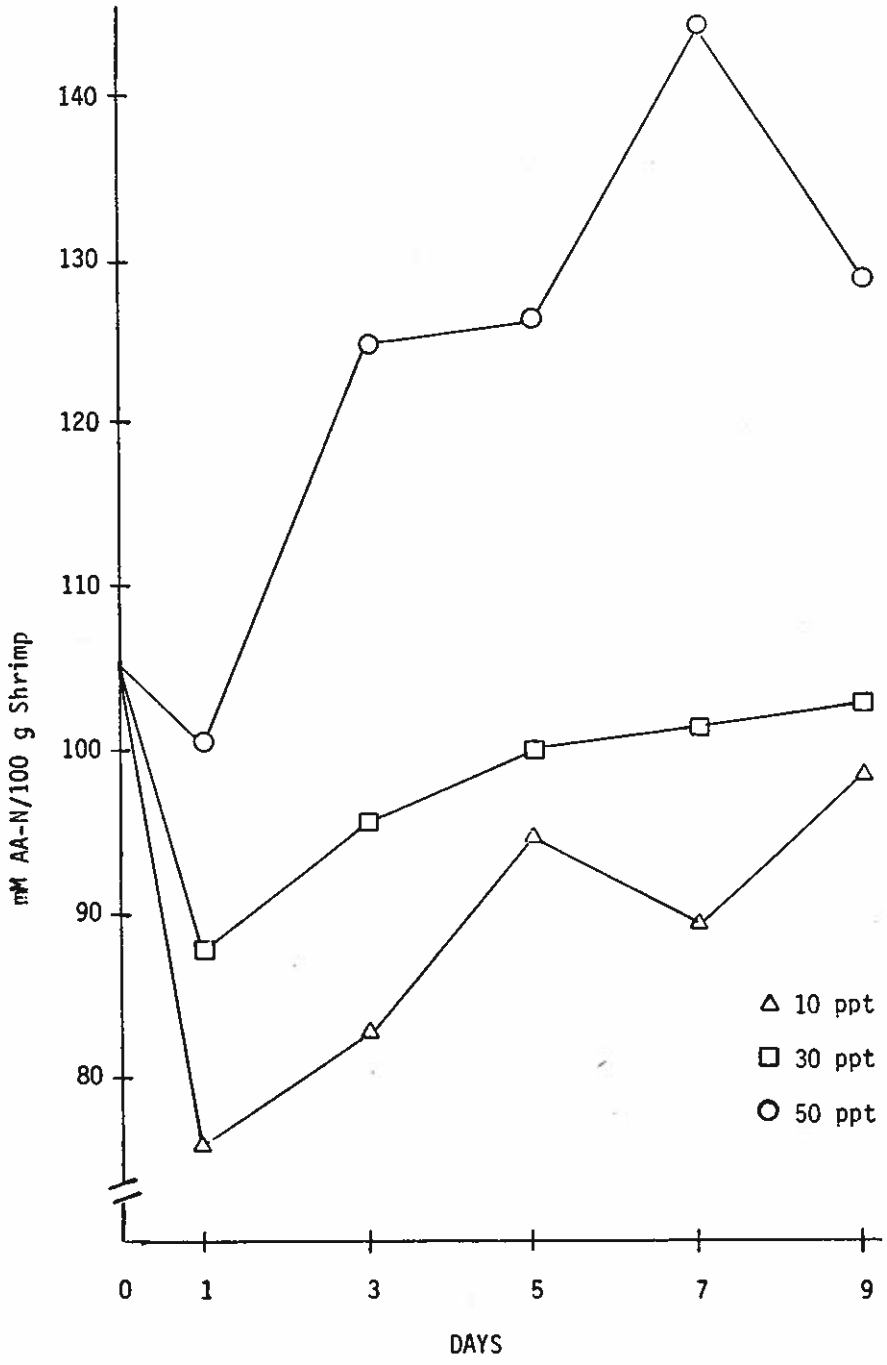


Figure 7. Amino acid nitrogen following a gradual salinity change - dry weight basis

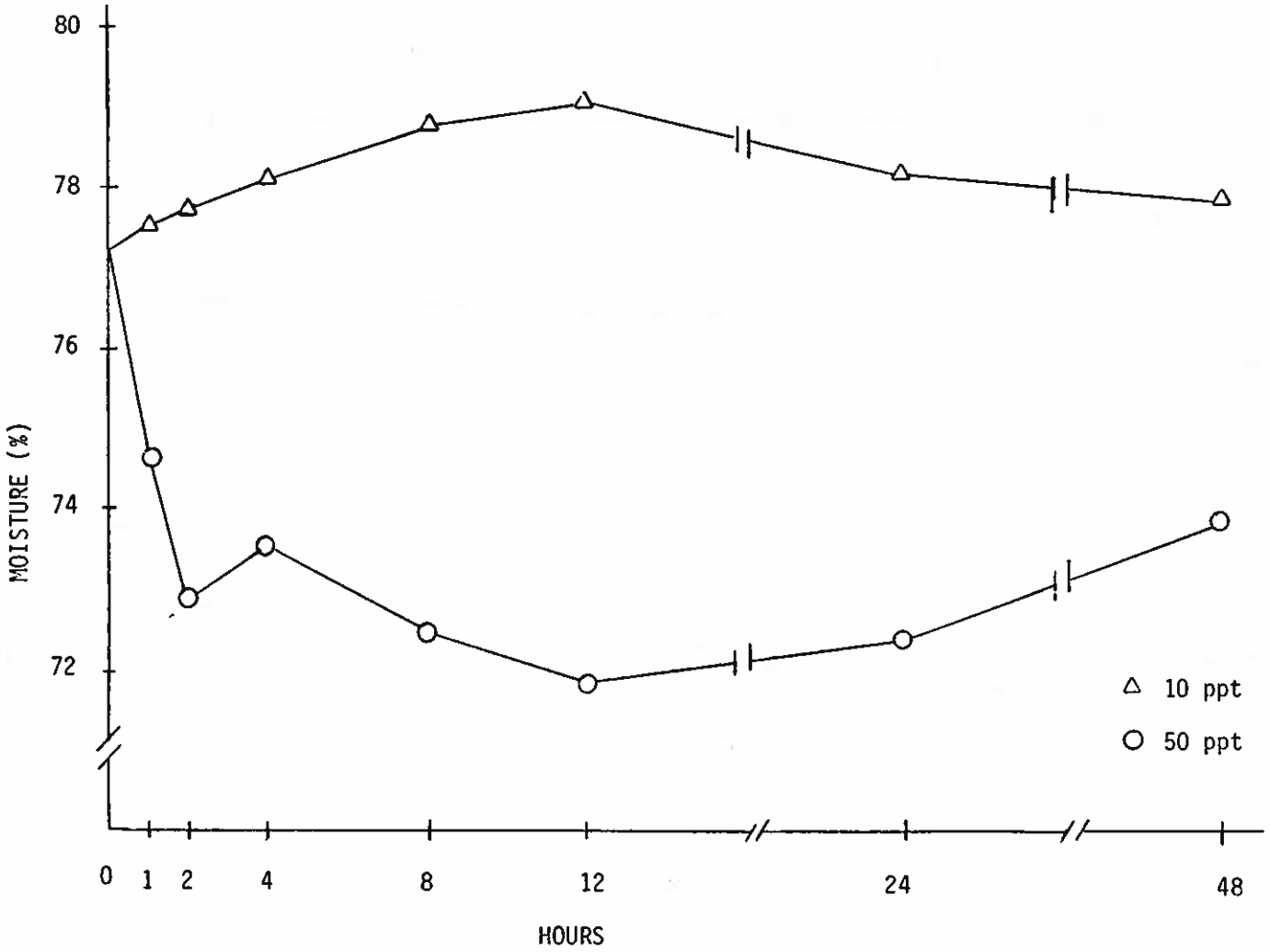


Figure 8. Moisture content following a simultaneous salinity change

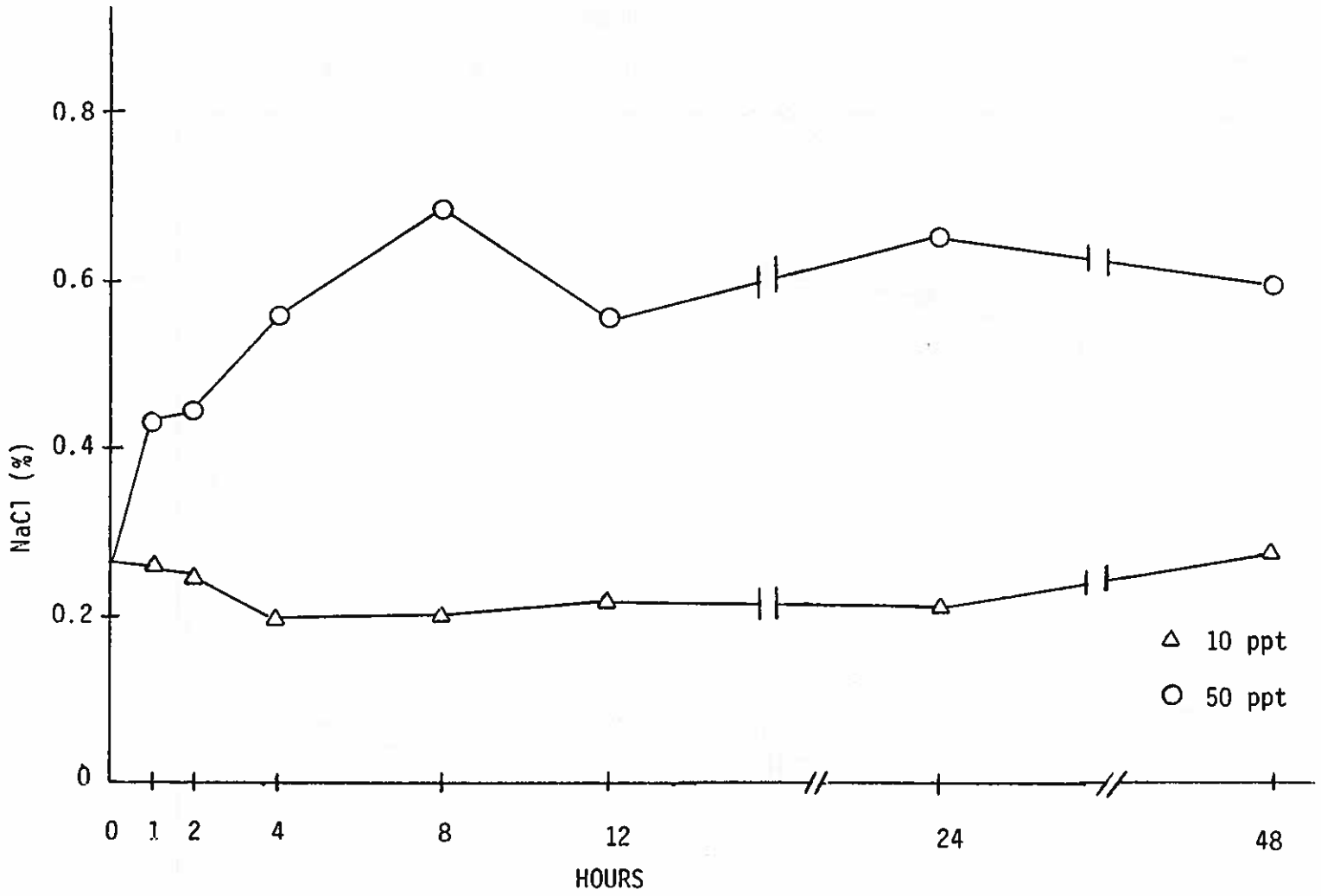


Figure 9. Salt content following a simultaneous salinity change - wet weight basis

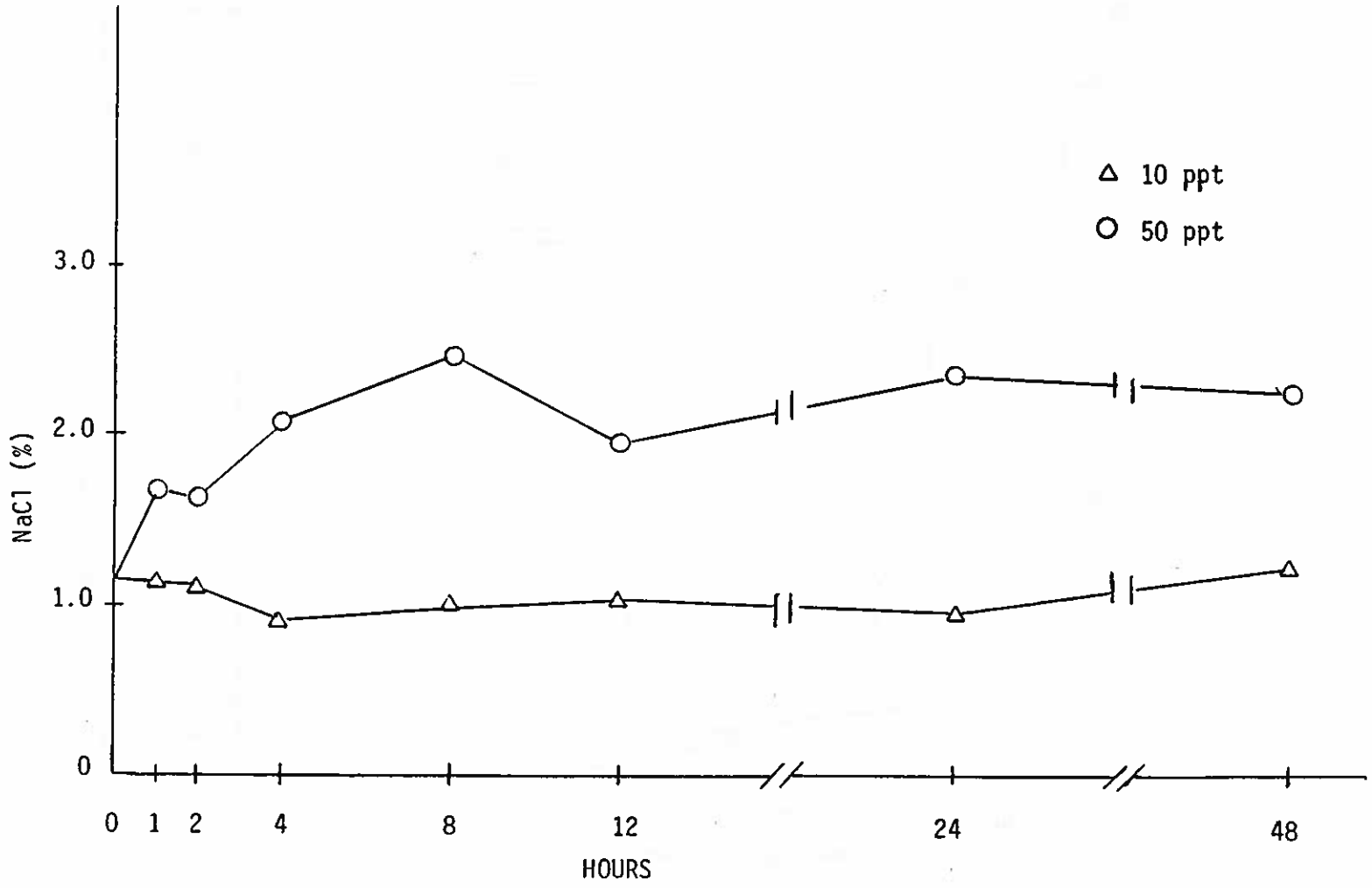


Figure 10. Salt content following a simultaneous salinity change - dry weight basis

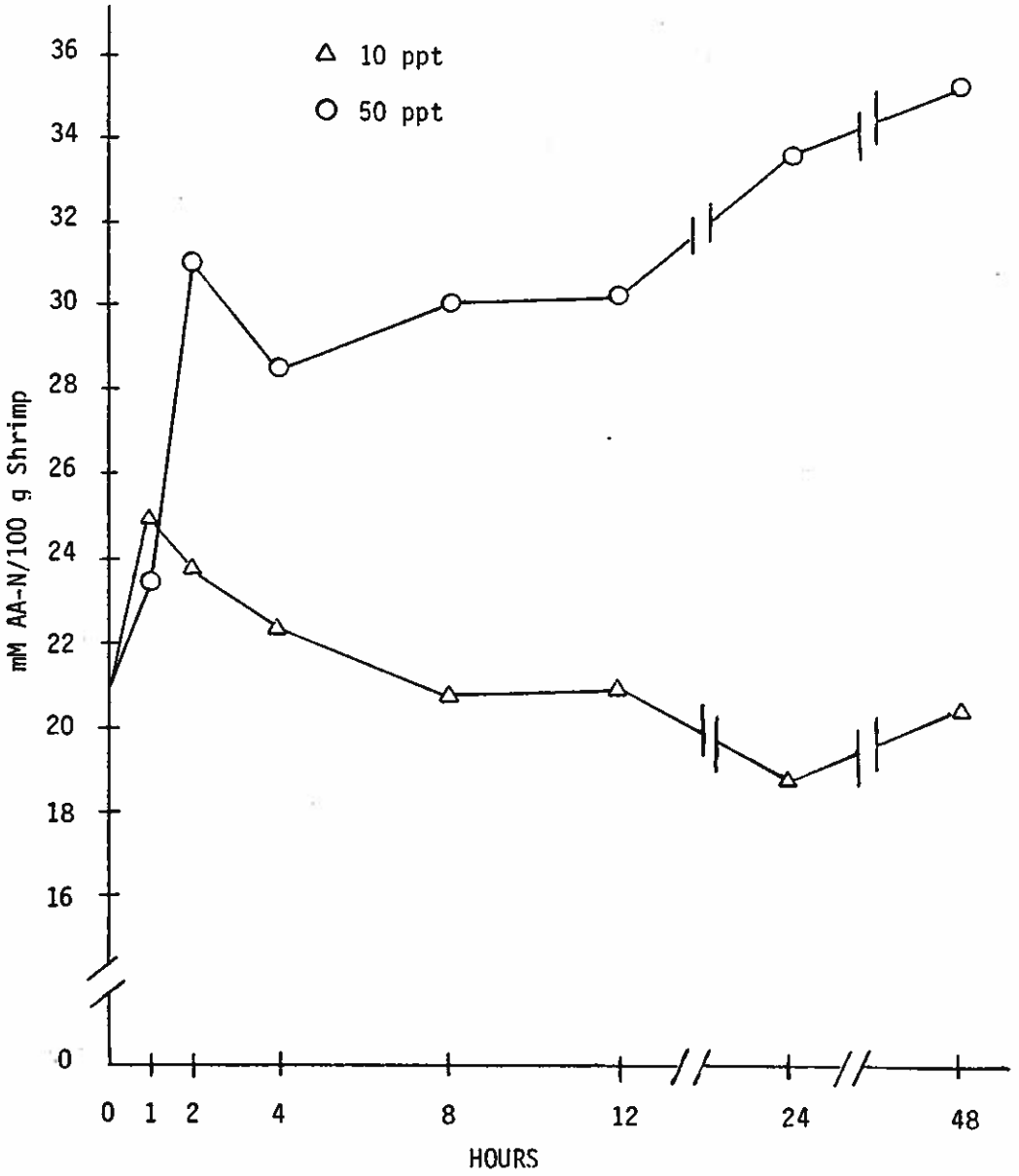


Figure 11. Amino acid nitrogen following a simultaneous salinity change - wet weight basis

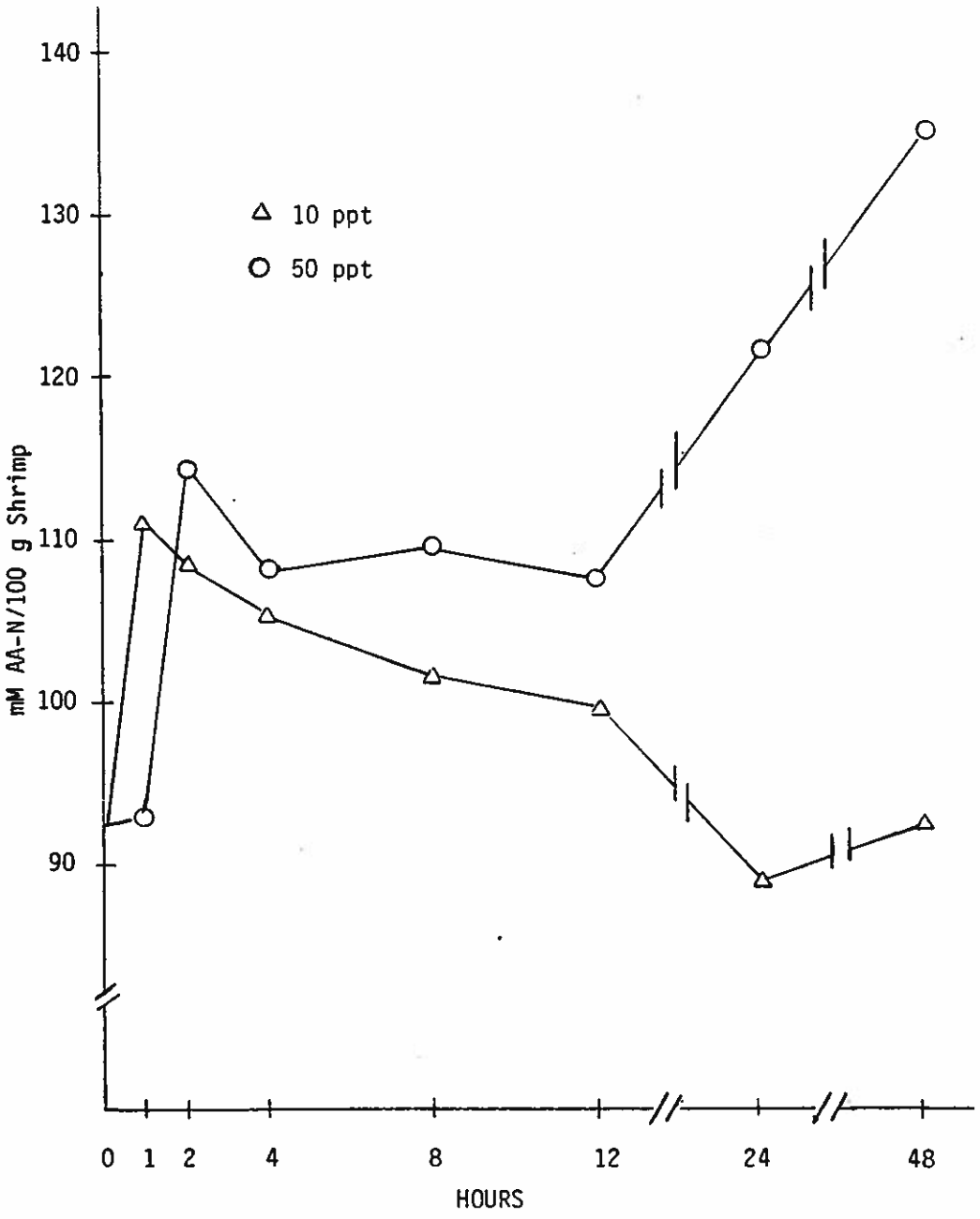


Figure 12. Amino acid nitrogen following a simultaneous salinity change - dry weight basis

THE EVALUATION OF THE EFFECTS OF THREE POST PROCESSING DIPS, 1% SODIUM BISULFITE, 100 PPM CALCIUM HYPOCHLORITE, AND 20 PPM CHLORINE DIOXIDE ON THE SHELFLIFE OF FRESH CALICO SCALLOPS

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INTRODUCTION

The recent rapid expansion of the calico scallop (Argopecten gibbus) industry from less than 5 million pounds processed in 1979 to greater than 15 million pounds processed in 1981 generated an intense interest in scallops among coastal Georgia seafood companies. Four new scallop plants were established in Georgia by the summer of 1982. Georgia processors, new to the business, requested advisory assistance from the Marine Extension Service. In addition to handling, sanitation, and quality control support, one processor requested that three post processing treatments be evaluated for their effects on the shelflife of fresh scallops. In the spring of 1983 a study was initiated to determine the effectiveness of three processing dips: (1) 1% sodium bisulfite, (2) 100 ppm calcium hypochlorite (HTH, Olin Corporation), and (3) 20 ppm chlorine dioxide (Odocine, ODCO Laboratories, Inc.) on the shelflife of scallops held on ice.

METHODS

The scallops (300 - 400 count) used in the study were caught off the coast of Cape Canaveral, Florida, on 20 March 1983, transported aboard trucks to Darien, Georgia, and processed by a commercial mechanical shucking line on 21 March 1983. Following shucking and inspection, the scallops passed through an iced brine tank that reduced the meat temperatures to 8.5°C. The scallops were drained and hand packed in 1 gallon (3.63 Kg) plastic containers. The contents of three separate containers were each dipped into 14 liters of one of the following solutions for 30 seconds: (1) 1% sodium bisulfite (pH = 5.13), (2) 100 ppm calcium hypochlorite (HTH) (pH = 9.51), or (3) 20 ppm chlorine dioxide (Odocine) (pH = 7.92). The scallops were drained, packed in fresh plastic containers and placed on ice. The iced containers were held in a refrigerator at 4°C for the duration of the study. The coolers containing the iced scallop containers drained continuously. Fresh ice was added as needed. An untreated control,

one gallon (3.63 Kg), container was also placed on ice. Small subsamples of the same lot of scallops were packed in Whirl-pak bags, frozen (-23°C) and used as sensory control samples for later organoleptic evaluations.

Scallop samples from the four containers: untreated, bisulfite dip, HTH dip, and Odocine dip, were evaluated chemically, microbiologically, and organoleptically at the end of 1, 2, 4, 7, 11, 14, and 16 days. All samples except the bisulfite treated and frozen control scallops were discarded following 16 days of storage. Panel members deemed the remaining samples organoleptically spoiled and unfit for additional evaluation. Bisulfite treated scallops continued to be sampled organoleptically through 18, 21, 25, 28, 30, 32, and 35 days of storage and through day 32 microbiologically. Frozen control samples were monitored through 30 days of storage before the supply was exhausted. Each sample was analyzed in duplicate for ammonium concentrations (11) and trimethylamine concentrations (2) and for pH levels. The following microbiological determinations were completed: aerobic plate count (7), fecal streptococci (enterococci) (10), MPN total coliforms (7), MPN total E. coli (7), and MPN coagulase positive staphylococci (7). A trained six member sensory panel evaluated each sample, including a frozen control for aroma and appearance. A modified aroma and appearance profile was used to characterize each scallop sample. A continuous sensory scale of 0 - 5 described each aroma or appearance characteristic. A score of zero indicated lack of detection by a panel member for a given trait while a score of five indicated the strongest impression for that trait (1,3, and 4). Additionally, each sample was evaluated for aroma and appearance on a consumer based continuous scale from 0 - 5, with a score of 5 indicating the greatest level of acceptance. The following aroma characteristics were defined:

(1) Briny smell: The aromatics associated with the smell of clean fresh seaweed and ocean air.
(2) Sweet: The sweet fragrance, minus the associated aromatics of many products, such as cooked fresh fish.
(3) Ammonia: The characteristic odor of the compound ammonia. A sharp irritation to the nostrils.
(4) Post Room Odor: The aroma associated with the viscera of freshly killed animals.
(5) Putrid: The aromatics associated with decaying fish or meat products.
(6) Sour: The aromatics associated with vinegar or lemon.
(7) Fishy: The aromatics associated with seafood that is getting off, but not yet old or spoiled. The smell of trimethylamine.
(8) Consumer Rating: A general evaluation of the product from a consumer's viewpoint. An excellent scallop in the freshest state would rate 5.

The following appearance characteristics were defined:

(1) Slimy: The amount of moist sticky substance coating the individual scallops.
(2) Light - Dark: The color of the scallop ranging from white (0) to grey (5).
(3) Firmness: The textural appearance and tactile sensation of the scallop. Zero indicated poor shape definition and a mushy feeling to the touch. Five indicated a well defined shape with a turgid appearance and a firm feeling to the touch.
(4) Adhesiveness: The tendency of individual scallops to clump together and stay that way. A score of 5 indicated a cohesive mass.
(5) Wetness: The amount of free moisture on the surface and drained from the scallops. Zero characterized a dry sample.
(6) Consumer Rating: A general evaluation of the product from a consumer's viewpoint. An excellent scallop rated 5.

All chemical, microbiological, and organoleptic data sets containing duplicate or greater than two values for each dependent variable were analyzed statistically with the Statistical Analysis System (SAS) (9). The methods included the General Linear Regression Model utilizing an analysis of variance procedure to compare the dependent variables for each day of storage with the three experimental and one control groups. Dependent variable means were compared for significant differences at the 0.05 level using Tukey's studentized range test (HSH) (9). Every treatment completed in duplicate was analyzed by a regression analysis on each dependent variable against time for the first 16 days of the storage study. Additionally, bisulfite scallop data were analyzed for each dependent taste panel variable over 35 days of storage and over 32 days of storage for the dependent chemical and microbiological variables. A significant correlation between a dependent variable and time was considered for probability values >F at the 0.05 level.

In the remainder of the paper, all significant differences will refer to a probability of $F > 0.05$ level. Significant differences between means will refer to Tukey's studentized range test, and significant regressions will refer to a standard linear regression model (9).

RESULTS AND DISCUSSION

Although several of the analyses and sensory panel descriptors provided significant differences or correlations, many failed to

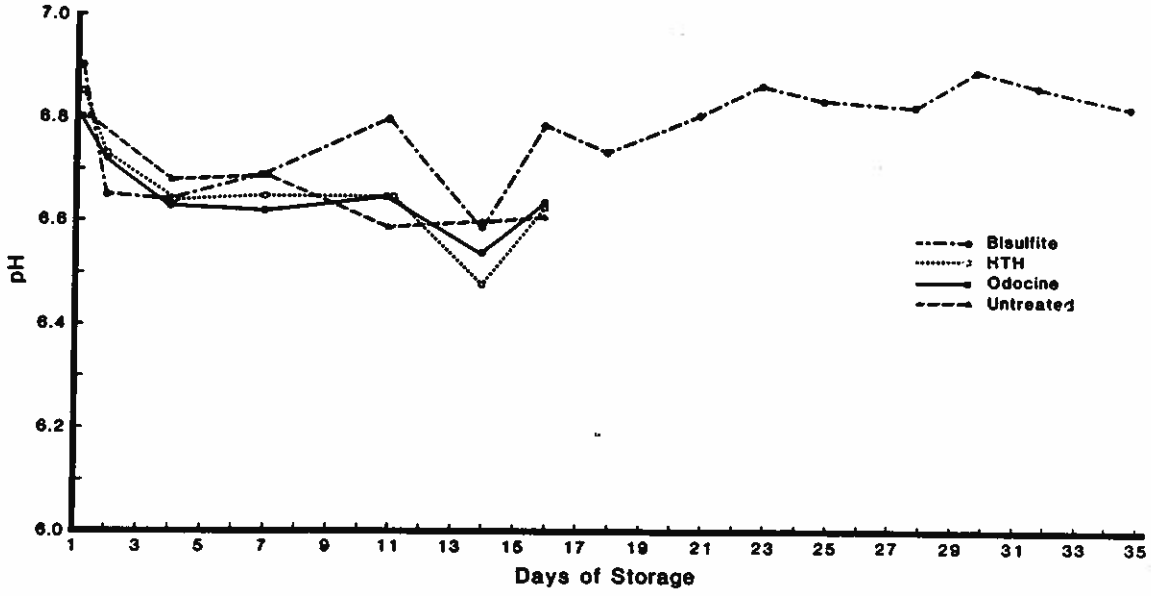

Figure 1. pH levels of HTH, Odocine, untreated, and bisulfite treated scallops.

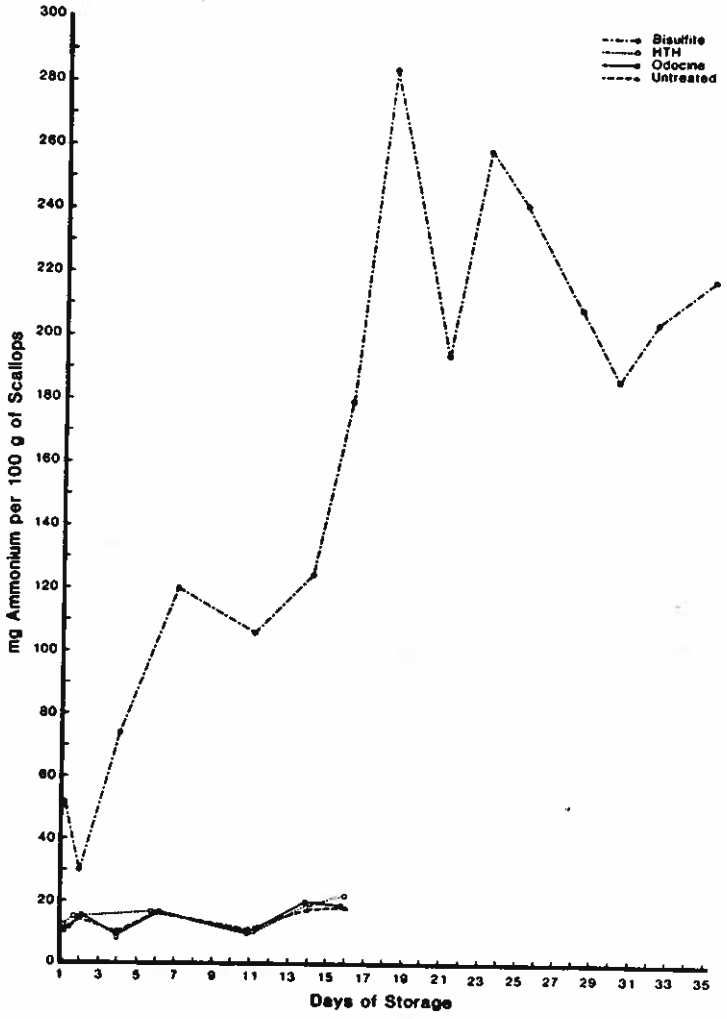

Figure 2. Mean ammonium levels in mg/100g for HTH, Odocine, untreated, and bisulfite treated scallops.

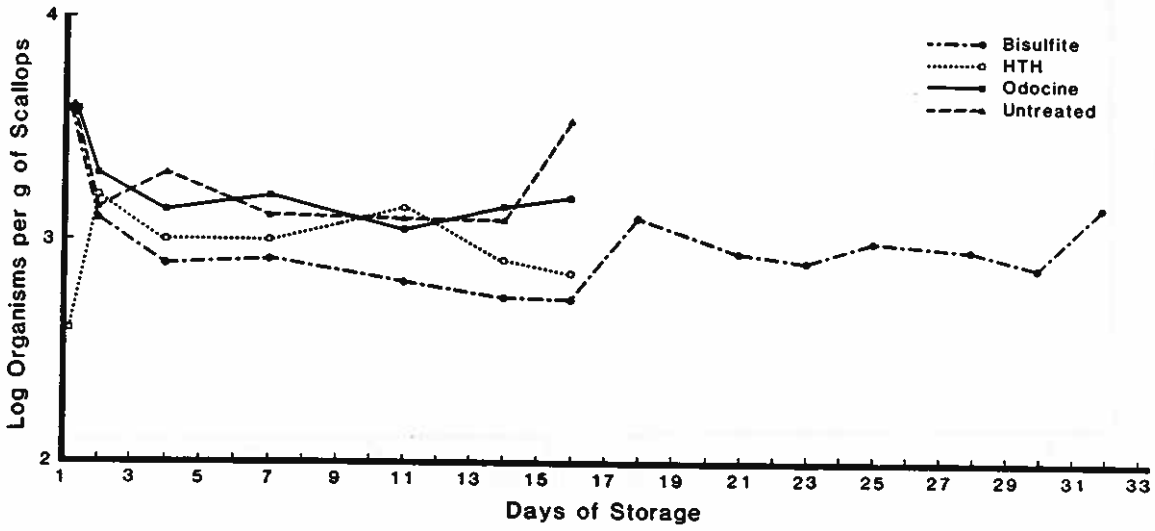

Figure 3. Fecal streptococci (enterococci) plate counts, organisms/g, for HTH, Odocine, untreated, and bisulfite treated scallops.

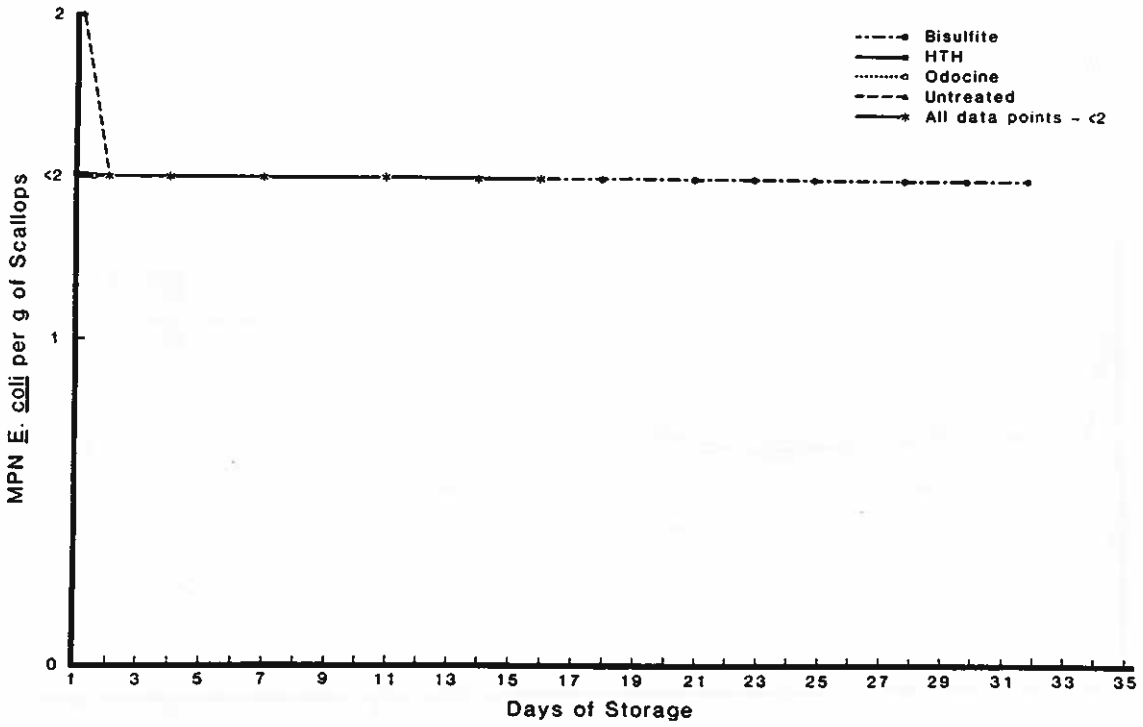

Figure 4. MPN E. coli organisms/g for HTH, Odocine, untreated, and bisulfite treated scallops.

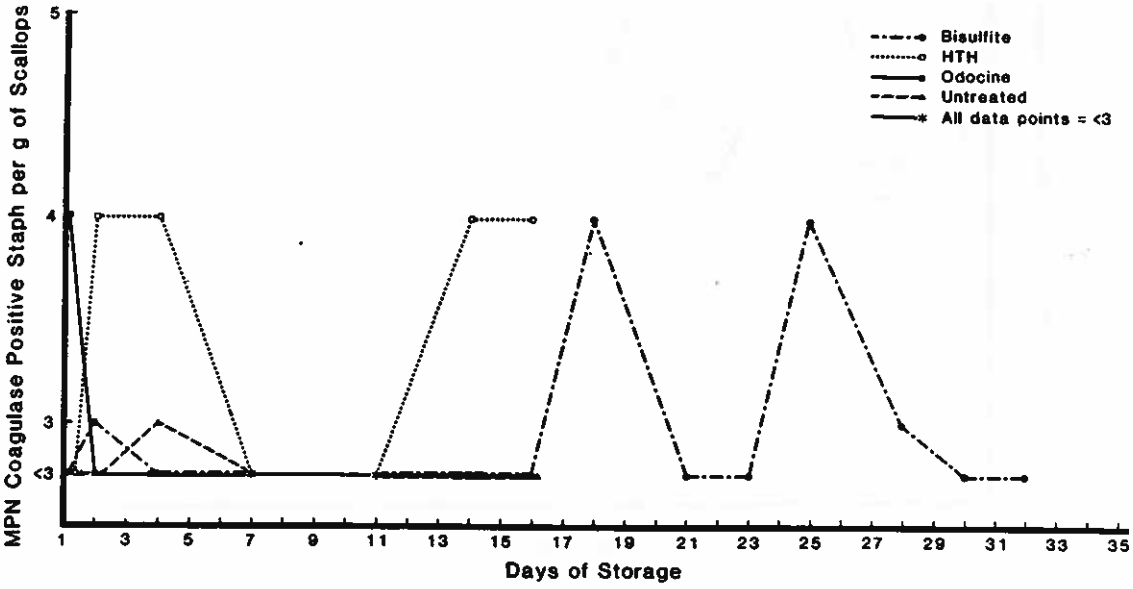

Figure 5. MPN coagulase positive staphylococci organisms/g for HTH, Odocine, untreated, and bisulfite treated scallops.

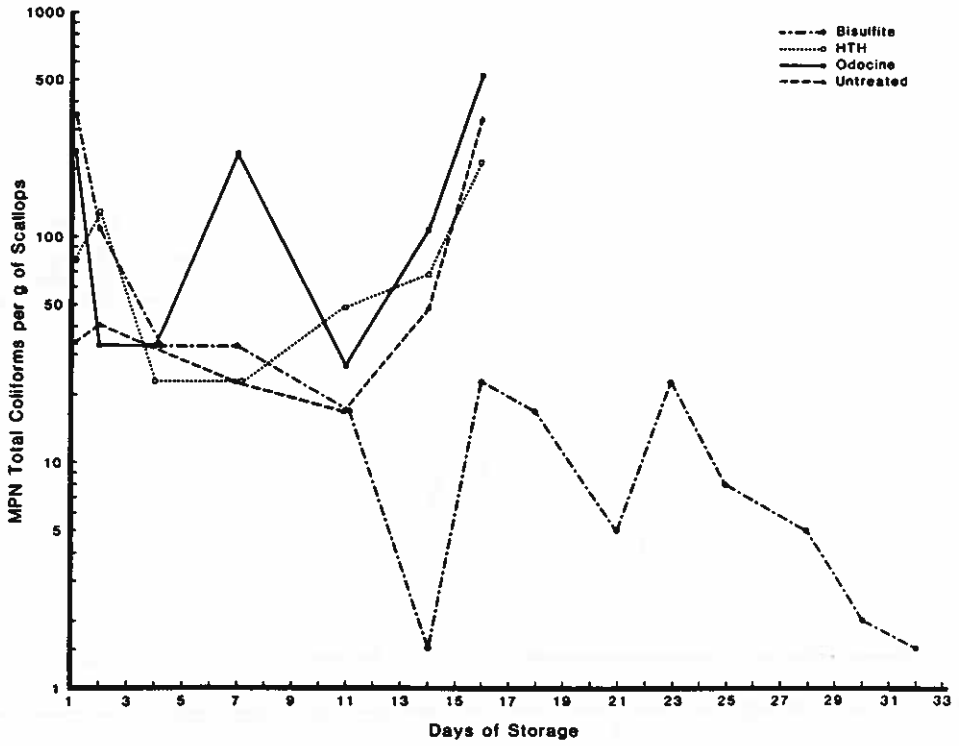

Figure 6. MPN total coliform organisms/g for HTH, Odocine, untreated, and bisulfite treated scallops.

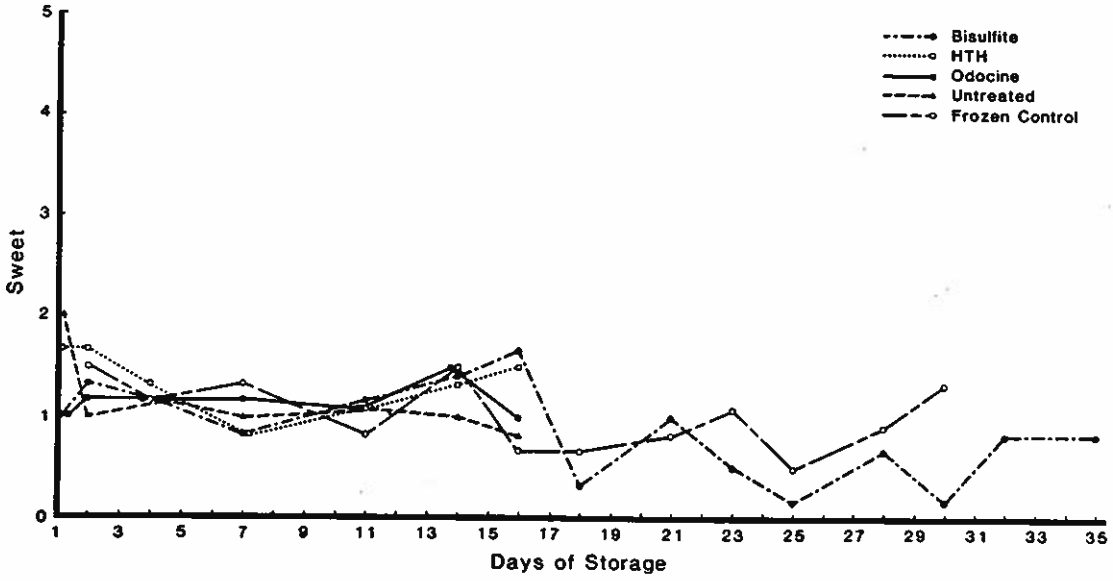

Figure 7. Mean sweet sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

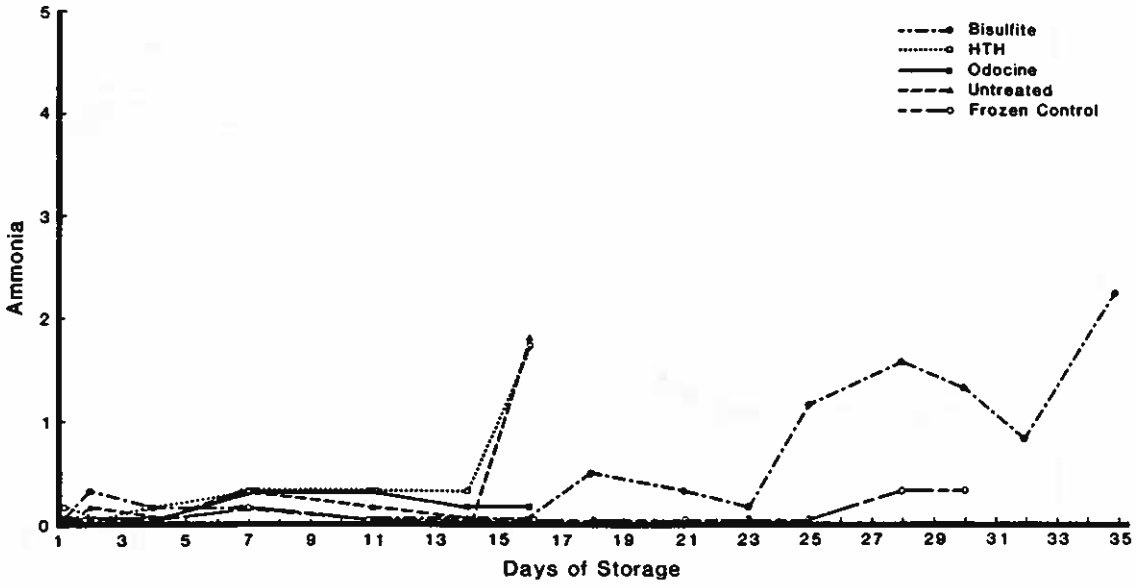

Figure 8. Mean ammonia sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

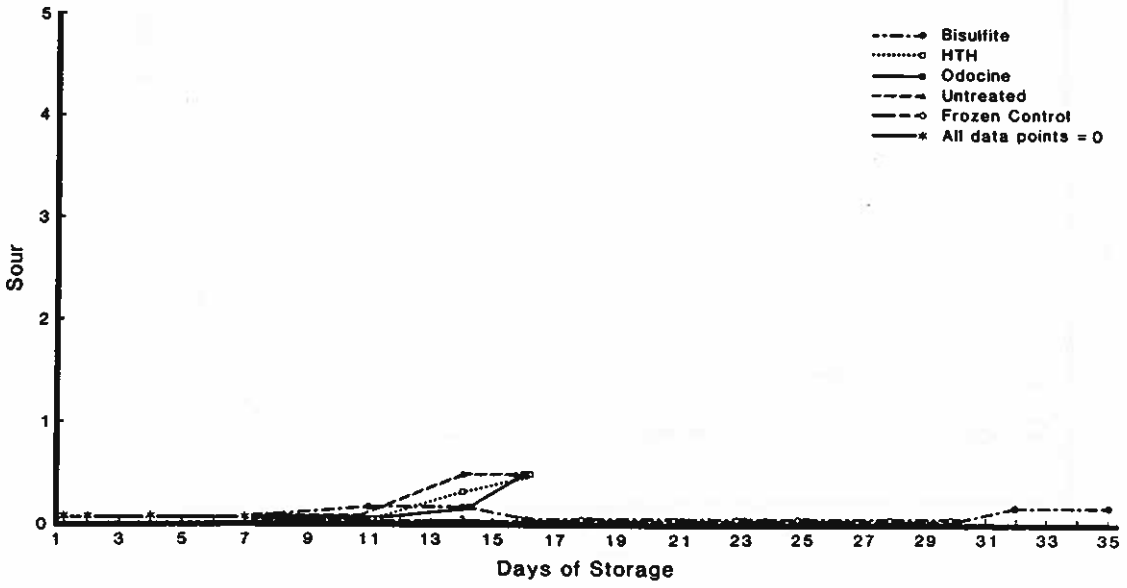

Figure 9. Mean sour sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

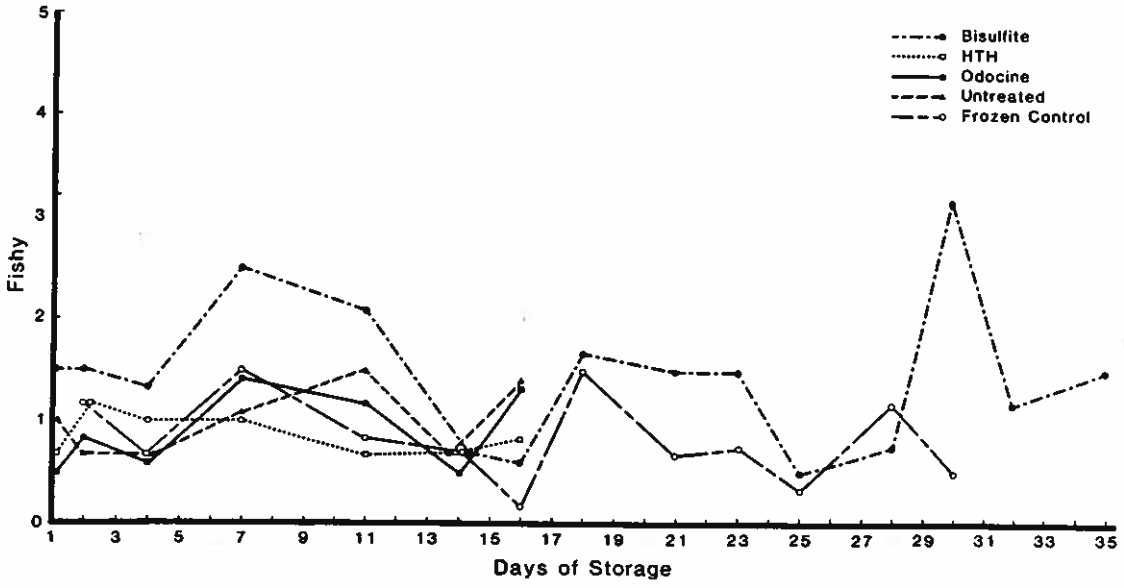

Figure 10. Mean fishy sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

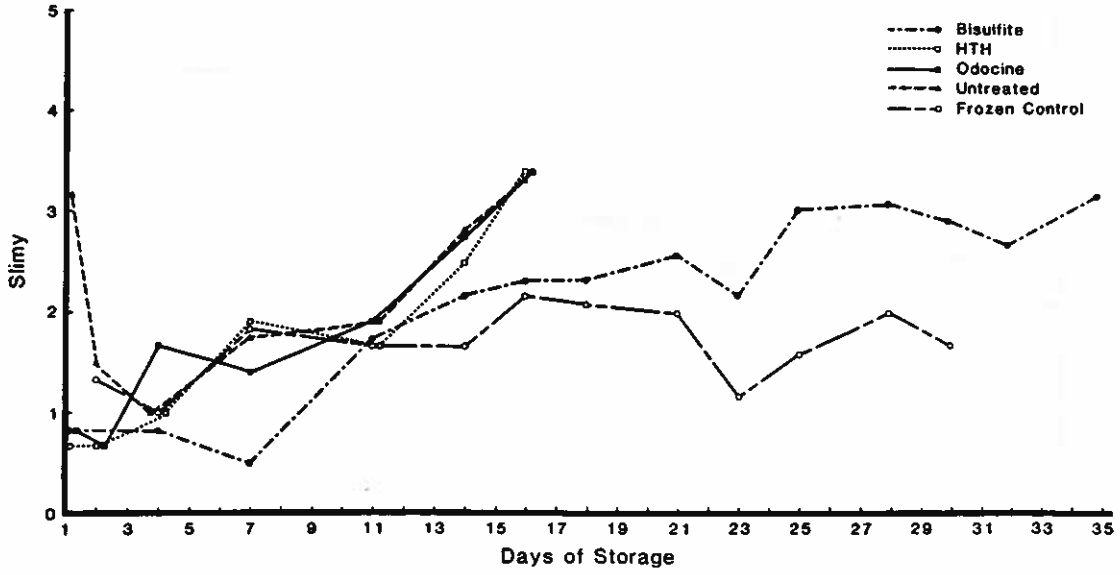

Figure 11. Mean slimy sensory scores for HTH, Odocine, untreated, bisulfite and frozen control scallops.

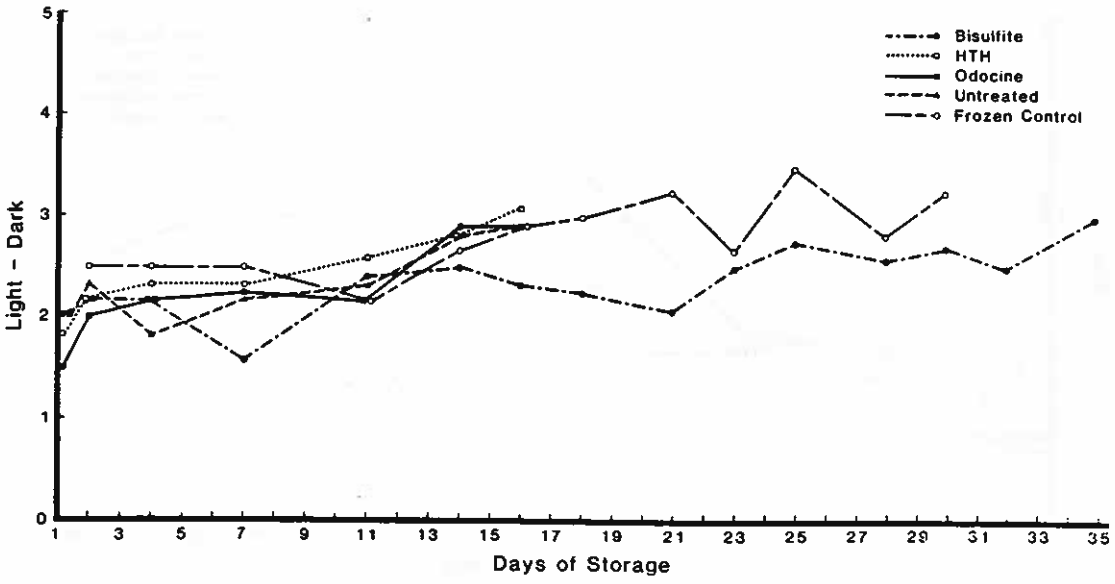

Figure 12. Mean light - dark sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

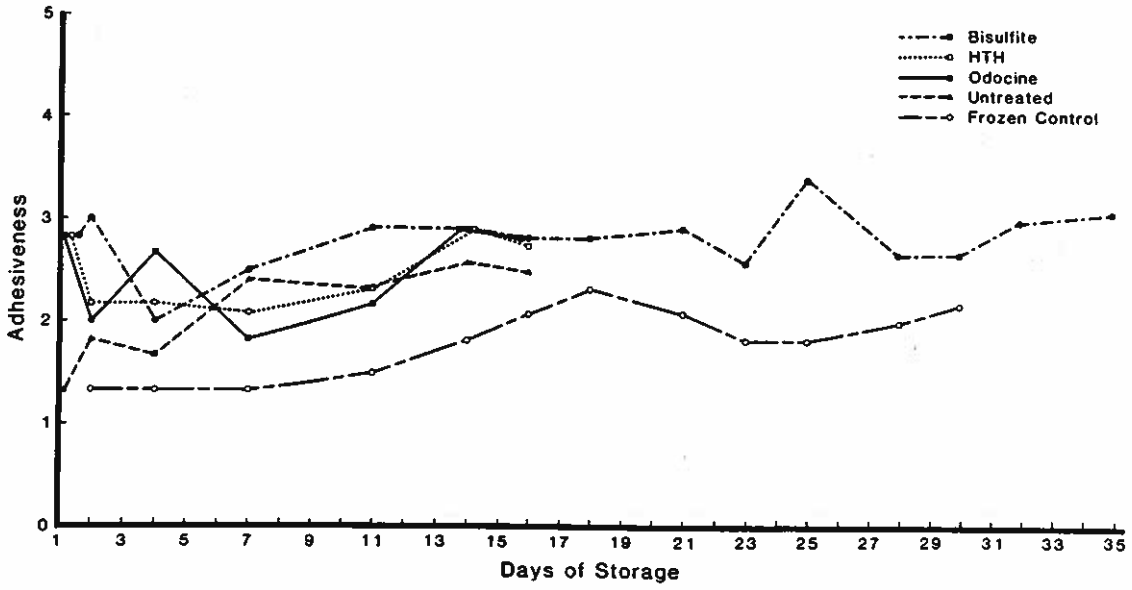

Figure 13. Mean adhesiveness sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

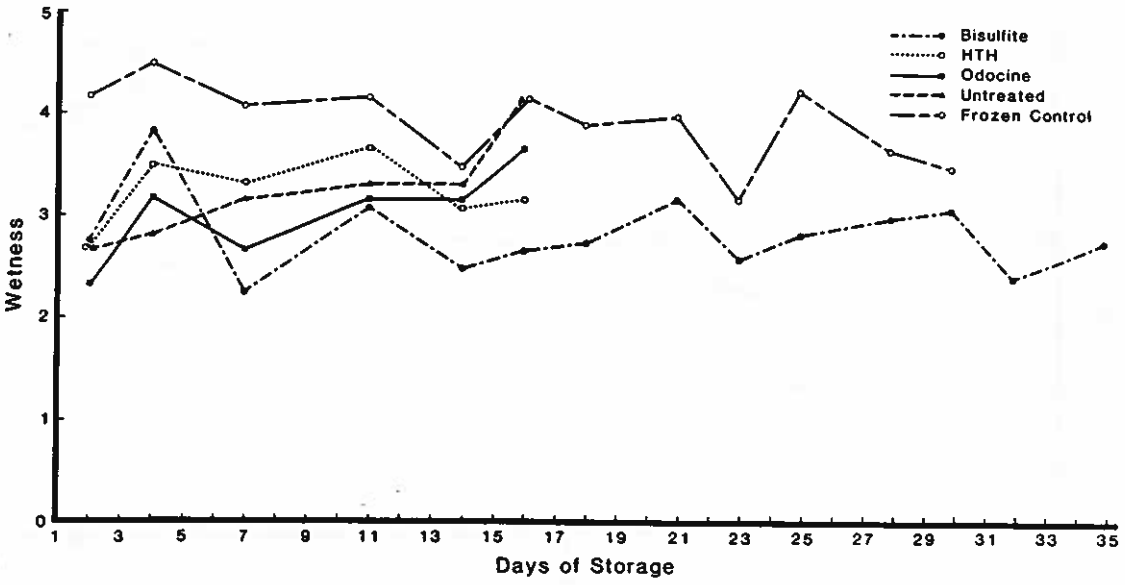

Figure 14. Mean wetness sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

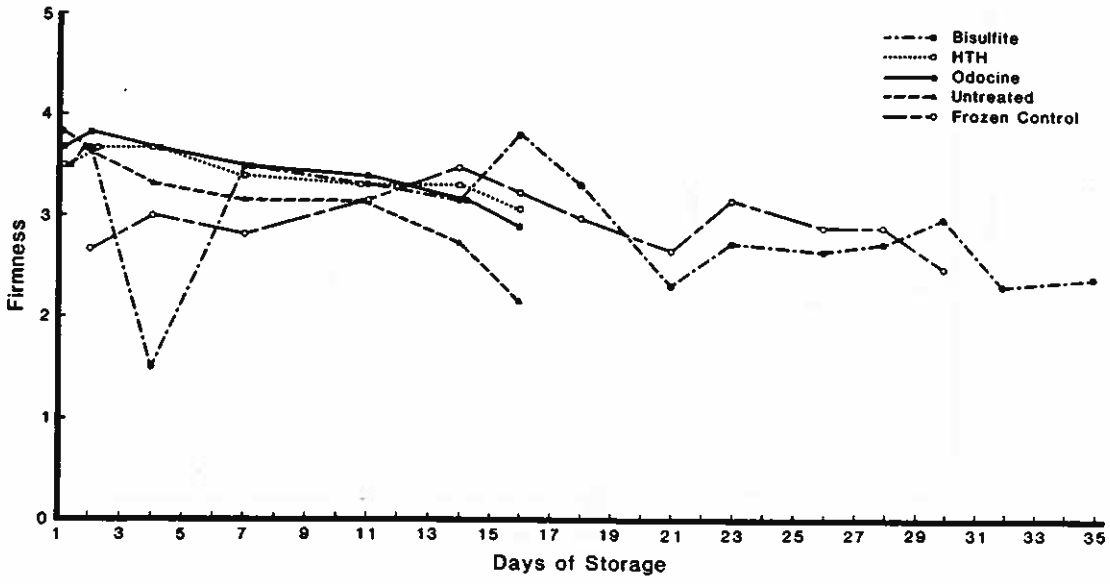

Figure 15. Mean firmness sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

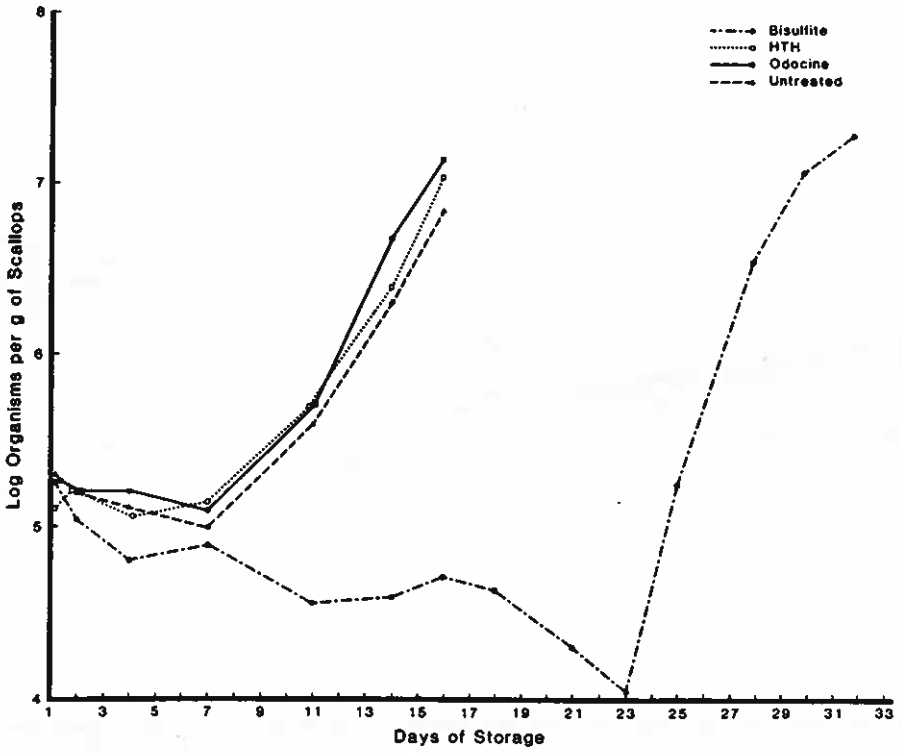

Figure 16. Mean aerobic plate counts, organisms/g, for HTH, Odocine, untreated, and bisulfite scallops.

differentiate scallop quality over time, or the effects of the various treatments. The following parameters fit into the above category.

The pH levels (Figure 1) of the experimental and control scallops were slightly greater than the values reported by Waters (12) over 16 days of storage. Waters' samples increased in pH with time from 6.55 - 6.65. The HTH, Odocine, and untreated samples decreased approximately 0.2 pH units over 16 days: 6.80 - 6.61, 6.85 - 6.63, and 6.80 - 6.64. The bisulfite scallops had an initial pH value of 6.90 and a final pH value of 6.83. The pH values did not serve as an effective indicator of spoilage.

Ammonium (Figure 2) levels determined for the bisulfite treated samples were greater than all other sample levels (significantly so on days 1, 4, 5, 11, and 14), but no correlation with quality was demonstrated (Table 1).

Fecal streptococci plate counts, MPN E. coli, or MPN coagulase positive staphylococci results failed to define product quality or show treatment differences (Figures 3, 4, and 5). Significant differences between fecal streptococci means were determined for days 1, 4, 7, 11, and 14. Bisulfite treated samples had significantly fewer fecal streptococci organisms than one or more of the other samples on days 4, 7, 11, and 14 (Table 2). MPN total coliform levels decreased from 348 to <2 organisms/g over 32 days of bisulfite scallop storage. The coliform levels of other samples increased with time, but all samples exceeded the FDA (5) guideline of an MPN = 23 organisms/g during the study (Figure 6).

Sensory evaluations of sweet (Figure 7), ammonia (Figure 8), sour (Figure 9), and fishy (Figure 10) odors failed to differentiate product quality or treatments (Table 3, 4, 5, and 6). The appearance characteristics of slimy (Figure 11), light - dark (Figure 12), adhesiveness (Figure 13) and wetness (Figure 14) did not prove useful (Tables 7, 8, 9, and 10). Although firmness (Figure 15) did not distinguish treatment or product quality following the iced storage of scallops, the significantly lower rating on day 4 for the bisulfite sample indicated a possible marketing problem (Table 11). The initial firmness rating fell from 3.67 to 1.50 by day 4, but returned to 3.50 by day 7.

Of the 22 monitored chemical, microbiological, and organoleptic parameters, only 7 proved useful in differentiating the quality of scallops during the storage study, aerobic plate count, TMA, briny odor, post room odor, putrid odor, consumer odor rating, and the consumer appearance rating.

Using FDA's (5) 500,000 (Log 5.70) aerobic plate count organisms/g guideline as a quality cut off standard for shellfish indicated a maximum shelflife of 11 days for the HTH and odocine samples, 12 days for the untreated samples, and 25 days for the bisulfite samples (Figure 16). Bisulfite scallop plate counts were significantly less

AMMOINUM

Days of Storage	Mean	Treatment
1	A 51.50	Bisulfite
	B 11.50	HTH
	B 11.40	Untreated
	B 10.45	Odocine
2	No significant difference	
4	A 74.00	Bisulfite
	B 10.35	Untreated
	B 9.30	Odocine
	B 8.50	HTH
7	A 120.00	Bisulfite
	B 16.50	HTH
	B 16.15	Odocine
	B 16.00	Untreated
11	A 106.50	Bisulfite
	B 11.50	Untreated
	B 10.15	HTH
	B 9.65	Odocine
14	A 125.00	Bisulfite
	B 19.95	Odocine
	B 19.50	HTH
	B 17.50	Untreated
16	No significant difference	
18	----------	
21	----------	
23	----------	
25	----------	
28	----------	
30	----------	
32	----------	
35	----------	

Table 1. Mean ammonium levels significantly different at the 0.05 level. Tukey's studentized range test. Means with the same letter are not significantly different.

FECAL STREPTOCOCCI

Days of Storage	Mean	Treatment
1	A 4.10×10^3	Untreated
	A 3.80×10^3	Bisulfite
	A 3.80×10^3	Odocine
	B 385	HTH
2	No significant difference	
4	A 2.01×10^3	Untreated
	BA 1.34×10^3	Odocine
	B 1.00×10^3	HTH
	B 770	Bisulfite
7	A 1.60×10^3	Odocine
	BA 1.33×10^3	Untreated
	B 1.02×10^3	HTH
	B 825	Bisulfite
11	A 1.37×10^3	HTH
	A 1.26×10^3	Untreated
	A 1.22×10^3	Odocine
	B 655	Bisulfite
14	A 1.43×10^3	Odocine
	BA 1.22×10^3	Untreated
	BA 805	HTH
	B 545	Bisulfite
16	No significant difference	
18	----------	
21	----------	
23	----------	
25	----------	
28	----------	
30	----------	
32	----------	
35	----------	

Table 2. Mean fecal streptococci levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

SWEET

Days of Storage	Mean	Treatment
1	A 2.00	Untreated
	BA 1.67	HTH
	B 1.00	Bisulfite
	B 1.00	Odocine
2	No significant difference	
4	No significant difference	
7	No significant difference	
11	No significant difference	
14	No significant difference	
16	A 1.67	Bisulfite
	BA 1.00	Odocine
	BA 0.83	Untreated
	BA 0.67	Frozen Control
	B 0.50	HTH
18	No significant difference	
21	No significant difference	
23	No significant difference	
25	No significant difference	
28	No significant difference	
30	A 1.33	Frozen Control
	B 0.17	Bisulfite
32	----------	
35	----------	

Table 3. Mean sweet levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

AMMONIA

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	No significant difference	
11	No significant difference	
14	No significant difference	
16	A 1.83	Untreated
	A 1.75	Odocine
	B 0.17	HTH
	B 0.00	Bisulfite
	B 0.00	Frozen Control
18	A 0.50	Bisulfite
	B 0.00	Frozen Control
21	No significant difference	
23	A 0.17	Bisulfite
	A 0.00	Frozen Control
25	A 1.17	Bisulfite
	B 0.00	Frozen Control
28	A 1.58	Bisulfite
	B 0.33	Frozen Control
30	A 1.33	Bisulfite
	B 0.33	Frozen Control
32	----------	
35	----------	

Figure 4. Mean ammonia levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

SOUR

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	No significant difference	
11	No significant difference	
14	No significant difference	
16	No significant difference	
18	No significant difference	
21	No significant difference	
23	No significant difference	
25	No significant difference	
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 5. Mean sour levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

FISHY

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	A 2.50	Bisulfite
	BA 1.50	Frozen Control
	BA 1.42	Odocine
	BA 1.08	Untreated
	B 1.00	HTH
11	A 2.08	Bisulfite
	BA 1.50	Untreated
	BA 1.17	Odocine
	BA 0.83	Frozen Control
	B 0.67	HTH
14	No significant difference	
16	A 1.42	Untreated
	BA 1.33	Odocine
	BA 0.83	HTH
	BA 0.50	Bisulfite
	B 0.17	Frozen Control
18	No significant difference	
21	No significant difference	
23	No significant difference	
25	No significant difference	
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 6. Mean fishy levels significantly different at 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

SLIMY

Days of Storage	Mean	Treatment
1	A 3.17	Untreated
	B 0.83	Bisulfite
	B 0.83	Odocine
	B 0.67	HTH
2	No significant difference	
4	No significant difference	
7	A 1.92	HTH
	BA 1.83	Frozen Control
	BA 1.75	Untreated
	BA 1.42	Odocine
	B 0.67	Bisulfite
11	No significant difference	
14	No significant difference	
16	No significant difference	
18	No significant difference	
21	A 2.58	Bisulfite
	B 2.00	Frozen Control
23	A 2.17	Bisulfite
	B 1.17	Frozen Control
25	A 3.03	Bisulfite
	B 1.58	Frozen Control
28	A 3.08	Bisulfite
	B 2.00	Frozen Control
30	A 2.92	Bisulfite
	B 1.67	Frozen Control
32	----------	
35	----------	

Figure 7. Mean slimy levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

LIGHT - DARK

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	A 2.50	Frozen Control
	BA 2.33	HTH
	BA 2.25	Odocine
	BA 2.17	Untreated
	B 1.58	Bisulfite
11	No significant difference	
14	No significant difference	
16	A 3.08	HTH
	BA 2.92	Frozen Control
	BA 2.92	Odocine
	BA 2.92	Untreated
	B 2.33	Bisulfite
18	A 3.00	Frozen Control
	B 2.25	Bisulfite
21	A 3.25	Frozen Control
	B 2.08	Bisulfite
23	No significant difference	
25	A 3.50	Frozen Control
	B 2.75	Bisulfite
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 8. Mean light-dark levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

ADHESIVENESS

Days of Storage	Mean	Treatment
1	A 3.83	Bisulfite
	A 2.83	HTH
	A 2.83	Odocine
	B 1.33	Untreated
2	A 2.67	Bisulfite
	BA 2.17	HTH
	BA 2.00	Odocine
	BA 1.83	Untreated
	B 1.33	Frozen Control
4	A 2.67	Odocine
	BA 21.7	HTH
	BA 2.00	Bisulfite
	BA 1.67	Untreated
	B 1.33	Frozen Control
7	A 2.50	Bisulfite
	A 2.42	Untreated
	BA 2.08	HTH
	BA 1.83	Odocine
	B 1.33	Frozen control
11	A 2.92	Bisulfite
	BA 2.33	HTH
	BA 2.33	Untreated
	BA 2.17	Odocine
	B 1.50	Frozen control
14	A 2.92	Bisulfite
	A 2.92	HTH
	A 2.92	Odocine
	BA 2.58	Untreated
	B 1.83	Frozen control
16	No significant difference	
18	No significant difference	
21	A 2.92	Bisulfite
	B 2.08	Frozen control
23	A 2.58	Bisulfite
	A 1.83	Frozen control
25	A 3.42	Bisulfite
	B 1.83	Frozen control
28	A 2.67	Bisulfite
	B 2.00	Frozen control
30	No significant difference	
32	----------	
35	----------	

Table 9. Mean adhesivness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

WETNESS

Days of Storage	Mean	Treatment
1	----------	
2	A 4.17	Frozen control
	B 2.75	Bisulfite
	B 2.67	HTH
	B 2.67	Untreated
	B 2.33	Odocine
4	A 4.50	Frozen control
	BA 3.83	Bisulfite
	BA 3.50	HTH
	B 3.17	Odocine
	B 2.83	Untreated
7	A 4.08	Frozen control
	BA 3.33	HTH
	BA 3.17	Untreated
	B 2.67	Odocine
	B 2.25	Bisulfite
11	No significant difference	
14	No significant difference	
16	A 4.17	Frozen control
	A 4.17	Untreated
	BA 3.67	Odocine
	BA 3.17	HTH
	B 2.67	Bisulfite
18	A 3.75	Frozen control
	B 2.75	Bisulfite
21	No significant difference	
23	No significant difference	
25	A 4.25	Frozen control
	B 2.83	Bisulfite
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 10. Mean wetness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

FIRMNESS

Days of storage	Mean	Treatment
1	No significant difference	
2	A 3.83	Odocine
	A 3.67	Bisulfite
	A 3.67	HTH
	A 3.67	Untreated
	B 2.67	Frozen control
4	A 3.67	HTH
	A 3.67	Odocine
	A 3.33	Untreated
	A 3.00	Frozen control
	B 1.50	Bisulfite
7	No significant difference	
11	No significant difference	
14	No significant difference	

Days of Storage	Mean	Treatment
16	A 3.83	Bisulfite
	BA 3.25	Frozen control
	BA 3.08	HTH
	BC 2.92	Odocine
	C 2.17	Untreated
18	No significant difference	
21	No significant difference	
23	No significant difference	
25	No significant difference	
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 11. Mean firmness levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

than the other sample populations on day 14 (Table 12). Bacterial growth moved into a logarithmic phase by day 11 for the HTH, Odocine, and untreated samples and by day 25 for the bisulfite samples.

The TMA data for the HTH, Odocine, and untreated scallops paralleled the aerobic plate count results, with a rapid increase in levels from day 11 to day 16, 6.86 - 36.65 mg/100g, 14.36 - 36.65 mg/100g, and 7.63 - 34.58 mg/100g (Figure 17). The bisulfite sample exhibited two TMA peaks, one at 16 days (49.65 mg/100g) that decreased to 17.44 mg/100g TMA on day 23, and increased to 43.71 mg/100g by day 35. The second peak reflected bacterial growth, the first did not. The results indicated enzymatic release of TMA followed by bacterial production. The TMA level determined for the bisulfite samples exceeded the levels determined for all other samples on all sample days and was significantly greater (Table 13) than the other samples on days 2, 4, and 7. Absolute TMA levels did not indicate scallop quality when the bisulfite treated scallops were compared to the remaining samples. Relative levels within each treatment group did serve as a significant quality indicator, however. Significant positive correlation existed for TMA and storage time for HTH ($r^2 = 0.420$), Odocine ($r^2 = 0.403$), and untreated ($r^2 = 0.604$) samples over 16 days of storage. Positive significant correlation coefficients were determined for bisulfite samples on days 16 ($r^2 = 0.564$) and 35 ($r^2 = 0.200$).

The sensory results from the briny odor determination were similar to the TMA and plate count data. HTH, Odocine, and untreated scallop briny scores decreased rapidly from day 11 to day 14 and from day 14 to day 16. Mean HTH, 1.83, 0.67, 0.17; Odocine, 2.00, 0.67, 0.17; and untreated, 2.50, 0.00, 0.17 scallop briny levels were significantly less than the bisulfite and frozen control samples on days 14 and 16 (Table 14). The bisulfite sample briny ratings decreased rapidly from day 25 (1.67) to day 35 (0.67) and were significantly less than the frozen control samples on days 18, 23, 28, and 30 (Table 14). Significant negative correlations were determined for HTH ($r^2 = 0.742$), Odocine ($r^2 = 0.764$), untreated ($r^2 = 0.680$) and bisulfite ($r^2 = 0.418$) samples over the storage period.

The rapid decline in HTH, Odocine, and untreated scallop quality between day 11 and 16 was mirrored by the post room odor scores. On days 11, 14, and 16, the odor scores were as follows: HTH, 0.83, 2.33, 2.42; Odocine, 0.50, 2.42, 3.33; and untreated, 0.50, 3.58, 3.33 (Figure 19). Bisulfite treated and frozen control samples had significantly lower post room odor ratings than the preceeding samples (Table 15). Significant positive regression correlations were obtained for HTH ($r^2 = 0.471$), Odocine ($r^2 = 0.292$), and untreated ($r^2 = 0.569$) samples for the first 16 days of storage. The bisulfite treated sample scores increased over 35 days of storage, 0 - 1.50. No quality break was discovered, however, the post room odor levels of the bisulfite scallops were significantly greater than the frozen control samples on days 4, 18, 23, and 28 (Table 15).

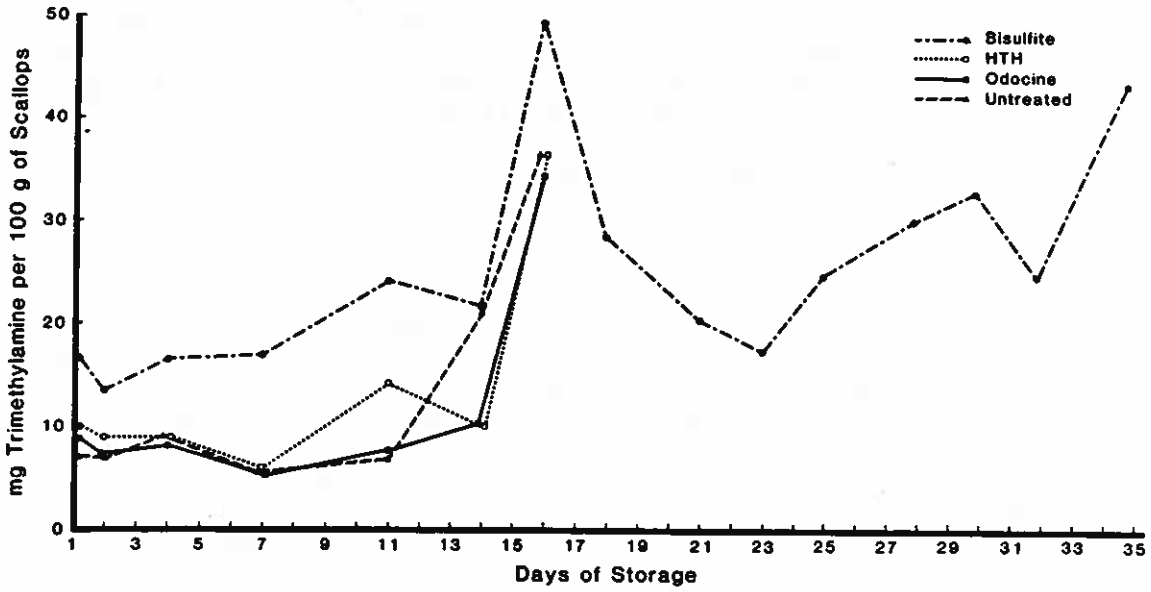

Figure 17. Mean trimethylamine levels, mg/100g, for HTH, Odocine, untreated, and bisulfite scallops.

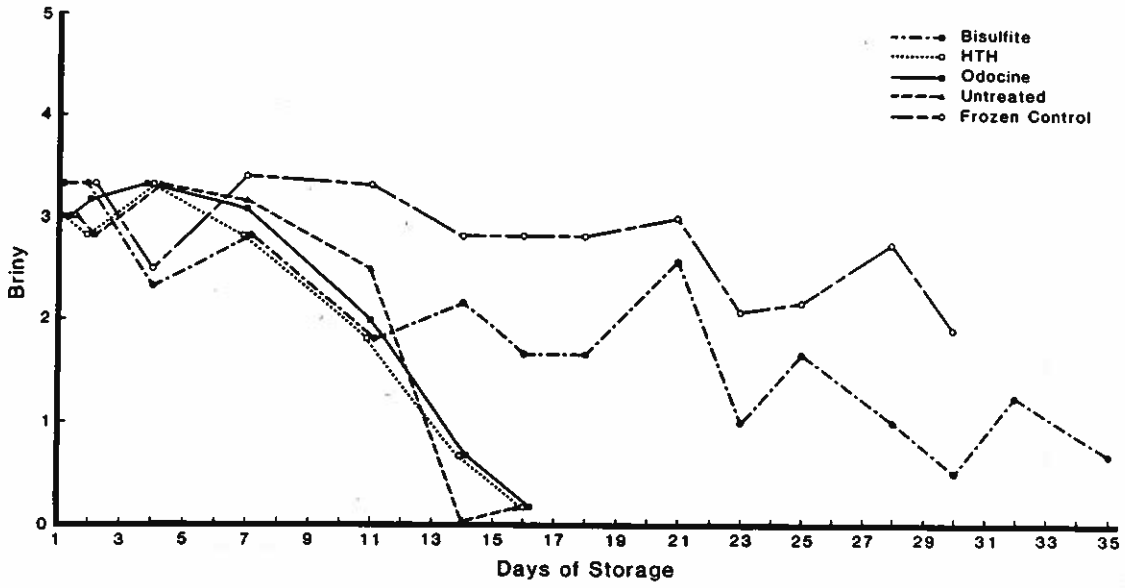

Figure 18. Mean briny sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

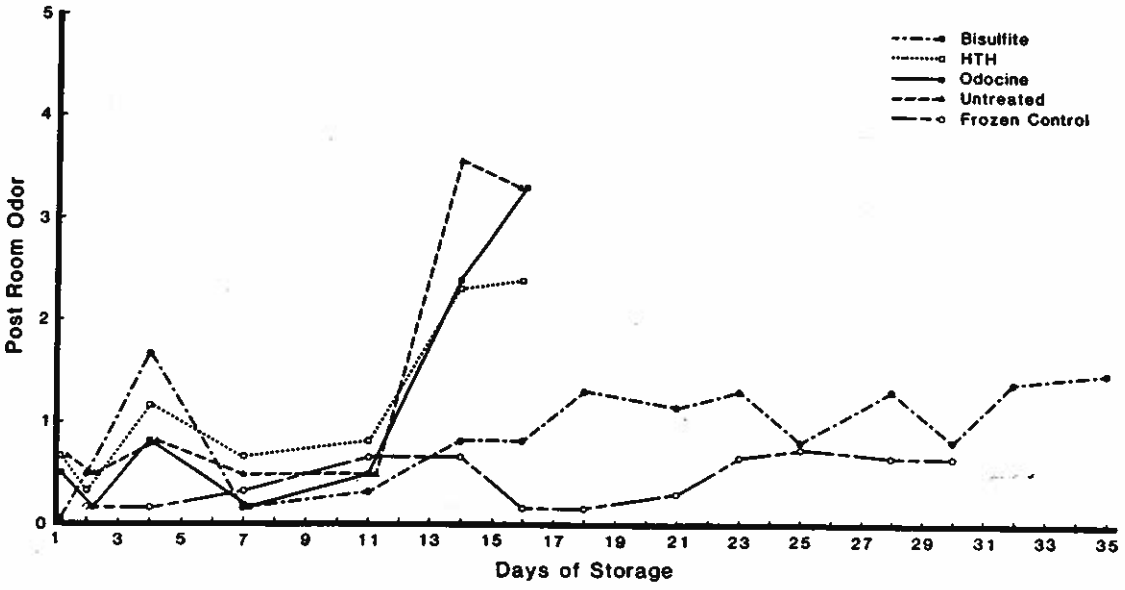

Figure 19. Mean post room odor sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

AEROBIC PLATE COUNT

Days of Storage	Mean	Treatment
1	A 2.06×10^5	Untreated
	A 1.84×10^5	Odocine
	A 1.84×10^5	Bisulfite
	B 1.29×10^5	HTH
2	No significant difference	
4	A 1.62×10^5	Odocine
	A 1.32×10^5	Untreated
	BA 1.17×10^5	HTH
	B 6.45×10^4	Bisulfite
7	A 1.36×10^5	HTH
	BA 1.24×10^5	Odocine
	BA 1.01×10^5	Untreated
	B 7.80×10^4	Bisulfite
11	A 5.30×10^5	Odocine
	A 5.15×10^5	HTH
	A 3.95×10^5	Untreated
	B 3.65×10^4	Bisulfite
14	No significant difference	
16	No significant difference	
18	----------	
21	----------	
23	----------	
25	----------	
28	----------	
30	----------	
32	----------	
35	----------	

Table 12. Mean aerobic plate counts significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

TRIMETHYLAMINE

Days of Storage	Mean	Treatment	Days of Storage	Mean	Treatment
1	A 16.67	Bisulfite	14	A 21.87	Bisulfite
	BA 9.98	HTH		A 21.28	Untreated
	B 8.75	Odocine		B 10.40	Odocine
	B 6.98	Untreated		B 10.28	HTH
2	A 13.48	Bisulfite	16	No significant difference	
	B 8.98	HTH			
	B 7.33	Odocine	18	----------	
	B 6.86	Untreated			
			21	----------	
4	A 16.55	Bisulfite			
	B 9.16	Untreated	23	----------	
	B 8.98	HTH			
	B 8.22	Odocine	25	----------	
7	A 17.14	Bisulfite	28	----------	
	B 6.08	HTH			
	B 5.62	Untreated	30	----------	
	B 5.32	Odocine			
			32	----------	
11	No significant difference				
			35	----------	

Table 13. Mean TMA levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

BRINY

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	A 3.33	HTH
	A 3.33	Odocine
	A 3.33	Untreated
	BA 2.50	Frozen control
	B 2.33	Bisulfite
7	No significant difference	
11	A 3.33	Frozen control
	BA 2.50	Untreated
	B 2.00	Odocine
	B 1.83	Bisulfite
	B 1.83	HTH
14	A 2.83	Frozen control
	A 2.17	Bisulfite
	B 0.67	HTH
	B 0.67	Odocine
	B 0.00	Untreated
16	A 2.83	Frozen control
	B 1.67	Bisfulfite
	C 0.17	HTH
	C 0.17	Odocine
	C 0.17	Untreated
18	A 2.83	Frozen control
	B 1.67	Bisulfite
21	No significant difference	
23	A 2.08	Frozen control
	B 1.00	Bisulfite
25	No significant difference	
28	A 2.75	Frozen control
	B 1.00	Bisulfite
30	A 1.92	Frozen control
	B 0.50	Bisulfite
32	----------	
35	----------	

Table 14. Mean briny levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

POST ROOM ODOR

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	A 1.67	Bisulfite
	BA 1.67	HTH
	BC 0.83	Odocine
	BC 0.83	Untreated
	C 0.17	Frozen control
7	No significant difference	
11	No significant difference	
14	A 3.58	Untreated
	A 2.42	Odocine
	A 2.33	HTH
	B 0.83	Bisulfite
	B 0.67	Frozen control
16	A 3.33	Odocine
	A 3.33	Untreated
	A 2.42	HTH
	B 0.83	Bisulfite
	B 0.17	Frozen control
18	A 1.33	Bisulfite
	B 0.17	Frozen control
21	No significant difference	
23	A 1.33	Bisulfite
	B 0.67	Frozen control
25	No significant difference	
28	A 1.33	Bisulfite
	B 0.67	Frozen control
30	No significant difference	
32	----------	
35	----------	

Table 15. Mean post room odor levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

PUTRID

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	No significant difference	
11	No significant difference	
14	A 1.83	Untreated
	BA 1.08	Odocine
	BC 0.83	HTH
	C 0.00	Bisulfite
	C 0.00	Frozen control
16	A 2.83	Untreated
	B 2.08	HTH
	B 1.83	Odocine
	C 0.00	Bisulfite
	C 0.00	Frozen control
18	No significant difference	
21	No significant difference	
23	No significant difference	
25	No significant difference	
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 16. Mean putrid levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

CONSUMER RATING, ODOR

Days of Storage	Mean	Treatment
1	No significant difference	
2	No significant difference	
4	No significant difference	
7	No significant difference	
11	A 4.00	Frozen control
	A 3.92	Untreated
	BA 3.83	HTH
	BA 3.75	Odocine
	B 3.25	Bisulfite
14	A 3.92	Frozen control
	BA 3.42	Bisulfite
	BC 2.58	Odocine
	BC 2.33	HTH
	C 2.08	Untreated
16	A 3.92	Frozen control
	A 3.42	Bisulfite
	B 1.83	Odocine
	B 1.42	HTH
	B 1.67	Untreated
18	A 4.00	Frozen control
	B 3.50	Bisulfite
21	A 3.33	Frozen control
	B 3.17	Bisulfite
23	A 3.33	Frozen control
	B 2.58	Bisulfite
25	A 3.50	Frozen control
	B 2.58	Bisulfite
28	A 3.67	Frozen control
	B 2.67	Bisulfite
30	A 3.42	Frozen control
	B 2.58	Bisulfite
32	----------	
35	----------	

Table 17. Mean consumer rating, odor levels significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

The putrid levels determined for all samples displayed a threshold response on day 14 for HTH, Odocine, and untreated samples and on day 30 for the bisulfite treated samples (Figure 20). For days 11, 14, and 16 the following responses were recorded: HTH, 0, 0.83, 2.08; Odocine, 0, 1.08, 1.83; and untreated, 0, 1.83, 2.83. On day 14 the odocine and untreated sample odor levels were significantly greater than the bisulfite or frozen control scallops (Table 16). On day 16 the bisulfite and frozen control samples had significantly lower scores than the other samples (Table 16). Putrid regression correlations were significant over 16 days storage for HTH ($r^2 = 0.546$), Odocine ($r^2 = 0.589$) and untreated ($r^2 = 0.625$) scallops. Bisulfite sample putrid odor ratings increased from 0 on day 28 to 0.67 by day 35.

The consumer aroma rating declined rapidly from day 11 through day 16 for the HTH, Odocine, and untreated samples (Figure 21). The aroma ratings on days 11, 14, and 16 were as follows: HTH, 3.83, 2.33, 1.42; Odocine, 3.75, 2.50, 1.83; and untreated 3.92, 2.08, 1.17. On day 14 the preceeding samples had significantly lower consumer odor ratings than the frozen control samples and by day 16 significantly lower scores than the frozen control and bisulfite samples (Table 17). Significant regression correlations were determined over 16 days of storage for HTH ($r^2 = 0.475$), Odocine ($r^2 = 0.661$), and untreated ($r^2 = 0.496$) scallops. The bisulfite sample scores declined over 35 days of storage with no sharp quality break, day 1 = 3.83, day 16 = 3.42, and day 35 = 1.88. The samples had significantly lower ratings than the frozen control scallops on days 23 - 30. A significant negative regression correlation was determined for 35 days of storage ($r^2 = 0.429$).

The consumer appearance rating exhibited the steepest decline for HTH, Odocine, and untreated samples between 11 and 16 days of storage (Figure 22). On days 11, 14, and 16, the following scores were recorded: HTH, 3.33, 3.00, 2.50; Odocine, 3.83, 2.75, 2.25; and untreated, 3.75, 2.58, 1.67. By day 16 the preceeding sample ratings were significantly less than the bisulfite treated scallops (Table 18). Significant regression correlations were determined over 16 days of storage for the HTH ($r^2 = 0.298$), Odocine ($r^2 = 0.583$), and untreated samples ($r^2 = 0.412$). Bisulfite samples decreased gradually with storage time from 4.33 (day 1) to 2.42 (day 35) and had a significant regression correlation over the 35 day period ($r^2 = 0.514$).

CONCLUSIONS

The treatment of fresh calico scallops (Argopecten gibbus) with three post processing dips, 1% sodium bisulfite, 100 ppm calcium hypochlorite (HTH), and 20 ppm chlorine dioxide (Odocine) resulted in useable iced shelflives of 25 days, 11 days, and 11 days, respectively. The experimental results compared with a 12 day shelflife for untreated scallops. Shelflife was arbitrarily defined as the time required for a sample to exceed an aerobic plate count of 500,000 organisms/g, the FDA shellfish guidelines (5, 6).

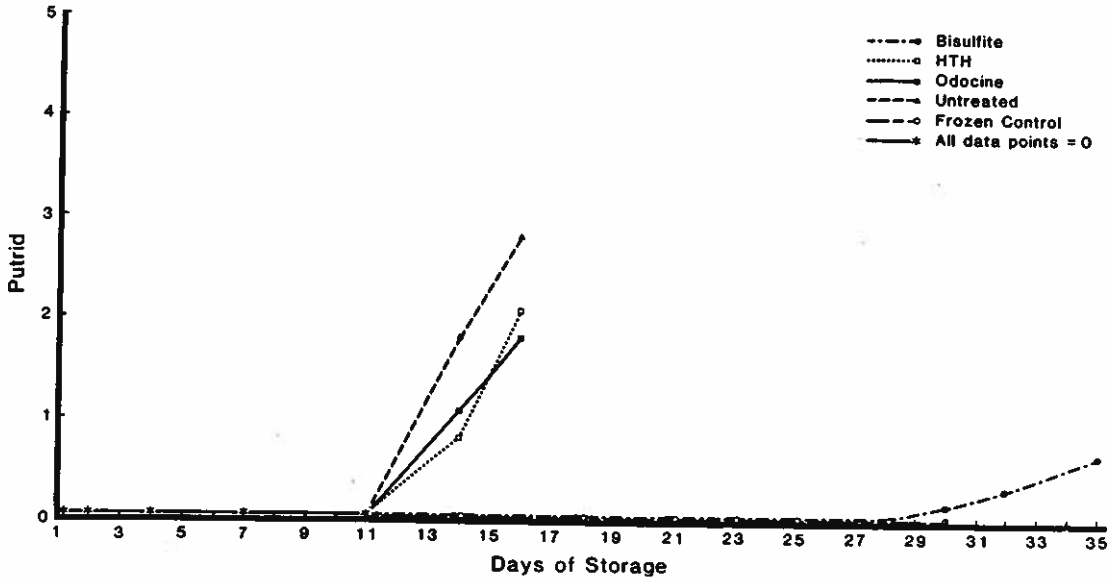

Figure 20. Mean putrid sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

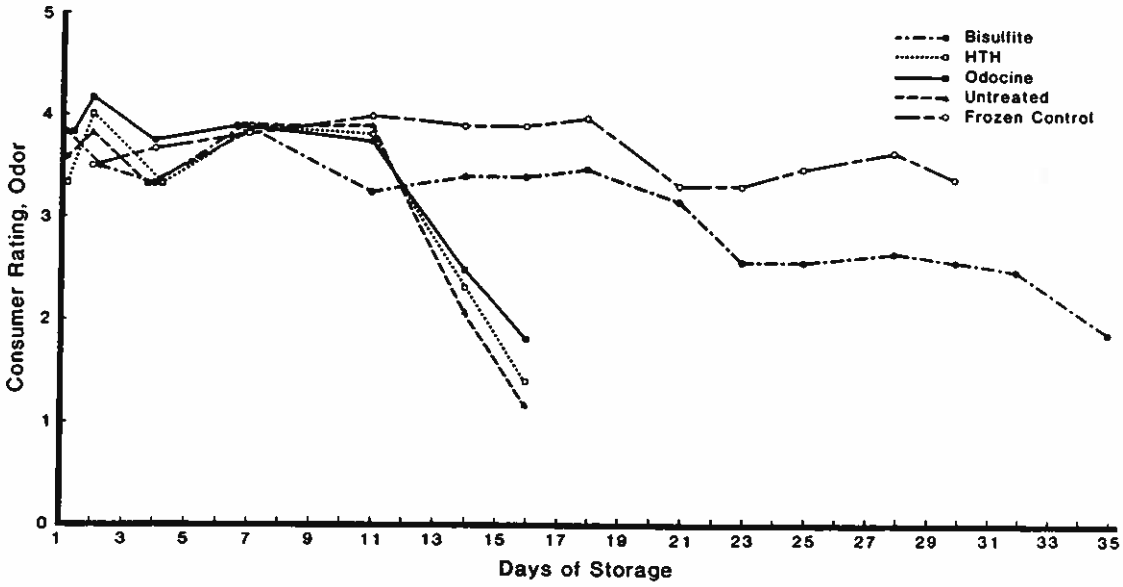

Figure 21. Mean consumer aroma sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

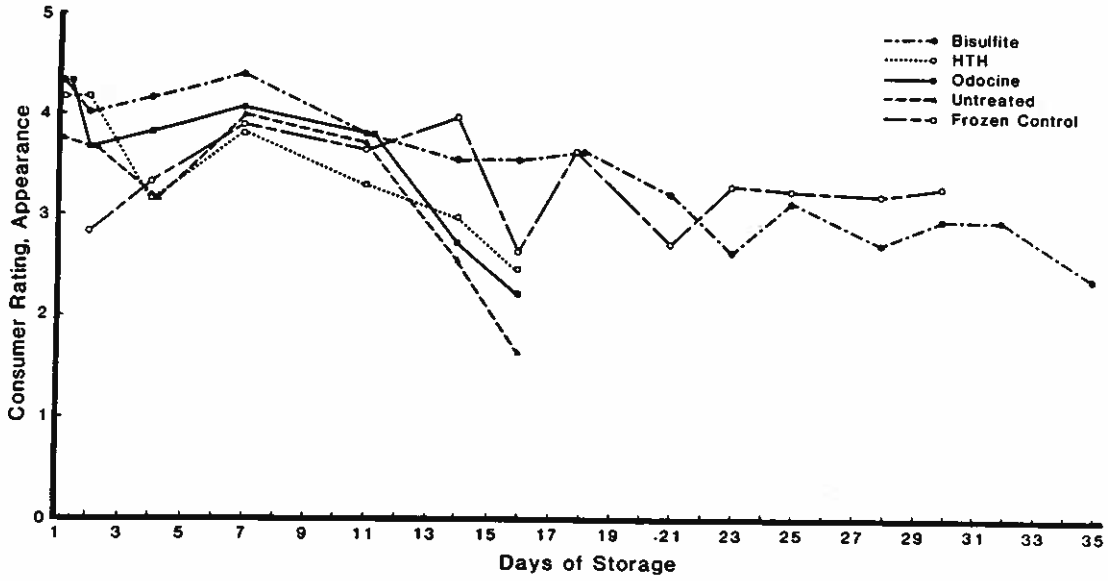

Figure 22. Mean consumer appearance sensory scores for HTH, Odocine, untreated, bisulfite, and frozen control scallops.

CONSUMER RATING, APPEARANCE

Days of Storage	Mean	Treatment
1	No significant difference	
2	A 4.33	Odocine
	A 4.17	HTH
	A 4.00	Bisulfite
	BA 3.67	Untreated
	B 2.83	Frozen control
4	No significant difference	
7	No significant difference	
11	No significant difference	
14	A 4.00	Frozen control
	BA 3.58	Bisulfite
	BA 3.00	HTH
	B 2.75	Odocine
	B 2.58	Untreated
16	A 3.58	Bisulfite
	BA 2.83	Frozen control
	BC 2.50	HTH
	BC 2.25	Odocine
	C 1.67	Untreated
18	No significant difference	
21	No significant difference	
23	A 3.33	Frozen control
	B 2.67	Bisulfite
25	No significant difference	
28	No significant difference	
30	No significant difference	
32	----------	
35	----------	

Table 18. Mean consumer ratings for appearance significantly different at the 0.05 level, Tukey's studentized range test. Means with the same letter are not significantly different.

Of the monitored chemical parameters, trimethylamine (TMA) levels proved to be a good quality indicator for HTH, odocine, and untreated scallops but not for bisulfite treated scallops. TMA levels rose rapidly following 11 days of storage for all samples. The bisulfite treated scallops exhibited two TMA peaks, one at 16 days and one at 35 days. The first peak probably represented enzymatic release of TMA, and the second, bacterial release (8). Although TMA levels were greater in the bisulfite treated scallops than the other samples, no sensory data correlated with the high TMA levels. Maximum TMA levels for all samples compared with the concentrations (> 50 mg/100g) determined by Waters (12) for untreated scallops.

Four of the odor characteristics monitored by the sensory panel proved to be good indicators of scallop quality when compared to aerobic plate counts. Briny, post room odor, putrid, and consumer odor ratings each expressed the rapid quality deterioration experienced by HTH, Odocine, and untreated scallop samples between 11 and 16 days of storage. Significant differences between the above samples and frozen control and/or bisulfite treated scallops were determined for each odor characteristic. Briny was the only odor characteristic that parallelled the rapid bacterial growth associated with the bisulfite scallops following 25 days of storage. Briny levels were significantly less than those determined for frozen control samples monitored at the same time.

Consumer appearance rating was the only visual characteristic that detected quality deterioration in the HTH, Odocine, and untreated scallops by the sixteenth day of storage. The ratings were significantly less than those determined for the bisulfite samples. The characteristics did not successfully determine quality deterioration in the bisulfite treated samples.

The treatment of calico scallops with a 1% sodium bisulfite dip for 30 seconds extended the product shelflife from 12 to 25 days. The scallops remained microbiologically and organoleptically acceptable for that period.

The bisulfite treatment provides an effective method to extend the shelflife of scallops that are not expected to reach the market within 10 - 14 days. However, the treatment did cause a significant decrease in firmness by the fourth day of storage. Firmness ratings returned to normal by the seventh day. The treatment should not be used on scallops that will be marketed within one week. Although bisulfite is listed as GRAS by FDA (6), residual bisulfite levels should be determined before the treatment is adopted by the scallop industry.

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THE POTENTIAL USE OF OZONATED ICE FOR ON BOARD STORAGE OF GULF OF MEXICO SHRIMP

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INTRODUCTION

Ozone is a powerful commercial oxidizing agent that has been used as a disinfectant for water supplies for over 70 years. Due to its antimicrobial properties, it has been studied as an aid in preservation of perishable foods. Farquhar and Rice (1982) reviewed the use of ozone in food preservation and reported that studies have been conducted on fruits, vegetables, eggs, cheeses, beef, poultry and fish. Neve (1982) studied the use of ozonated ice to extend the shelf life of fresh Alaskan fish. He found that the shelf life could be extended up to six days by using ice that contained 0.5 ppm ozone and that the bacterial counts and decomposition products were significantly reduced.

The shelf life of ice-stored shrimp aboard fishing vessels is directly related to the number and type of bacteria associated with the product (Nickelson and Vanderzant, 1976). Under typical spoilage, a shift in flora from predominantly gram-positive to gram-negative is observed. The objectives of this study were to: (1) evaluate the numbers and types of microorganisms on shrimp stored on ozonated ice, (2) to relate any changes to standard chemical analysis related to quality and (3) to assess the oxidizing potential of ozone on the development of melanosis.

MATERIAL AND METHODS

This study was conducted in two phases. In the first phase, shrimp were purchased on the coast and brought to the Food Quality and Safety Laboratory in College Station where they were stored on ozonated ice produced in Tyler (Howe Baker Engineers, Inc) and delivered to College Station. The second phase was conducted at the Seafood Technology Pilot Plant at the Texas Agricultural Experiment Station in Corpus Christi. The shrimp in this phase were purchased in the Corpus Christi area and the ozonated ice was produced in the lab

at Corpus Christi. These shrimp were also pre-rinsed in ozonated solutions prior to storage on ozonated ice.

College Station Phase

Approximately 100 pounds of bay shrimp (heads on) were obtained from Evelyn's Fish Market in Port Lavaca and transported to College Station where they were headed, rinsed and sorted into five treatment groups. Each treatment was stored in a separate 48 quart Igloo cooler with a false bottom. The treatments were stored on one of three types of ice: (1) normal ice, (2) ice made from water with a high ozone concentration (ca 2.0 ppm) and (3) ice made from water with a low ozone concentration (ca 0.5 ppm). The five treatments in this study were: (1) Control-#1 ice storage, (2) High Ozone-#2 ice storage, (3) Low Ozone-#3 ice storage, (4) High Ozone Bisulfite-bisulfite rinse followed by #2 ice storage and (5) Low Ozone Bisulfite-bisulfite rinse followed by #3 ice storage.

Shrimp from each group were tested on days 0, 1, 3, 5, 7, 9, 11, 13, 15 and 17. On each test day, approximately 400 grams of shrimp were removed from each treatment and tested for (1) aerobic plate count, (2) distribution of gram-positive versus gram-negative organisms, (3) pH, (4) black spot, (5) total volatile nitrogen and (6) ammonia. The thaw drip was also collected from the coolers as needed and recorded. The shrimp were re-iced on days 4 and 11.

Aerobic plate counts (APC) were obtained by combining 50 grams of shrimp with 450 ml of peptone dilution water and spread plating serial dilutions. Duplicate samples were done for each treatment group. Blending was accomplished using a Stomacher 400 Lab Blender. The distribution of gram-positive versus gram-negative organisms was obtained by gram staining organisms from duplicate plates of approximately 30-60 organisms. After initial typing of organisms, the remainder of the distributions were done by sight identification by color and colony appearance.

The pH was obtained using a surface probe on 5 randomly selected shrimp from each group. The pH was taken on two areas of each shrimp, the segment where the head was removed and the segment approximately halfway to the tail. Black spot was determined on 25 shrimp from each treatment. The percent of black spot was recorded as was the percent of surface covered. Total volatile nitrogen was determined using the Conway microdiffusion procedure of Cobb et al. (1973). Ammonia was determined using an ammonia probe according to the procedure of Ward et al. (1979).

Corpus Christi Phase

Fresh bay shrimp were obtained from Bandit Seafood in Port Aransas, transported to the laboratory in Corpus Christi and deheaded. Two samples of approximately 50 grams each were removed to determine initial bacterial levels. The remaining shrimp were divided into the following treatments: (1) Control-sea water rinse, shaved ice storage, (2) Fresh Water-ozonated fresh water rinse, ozonated ice storage, (3)

Sea Water-ozonated sea water rinse, ozonated ice storage and (4) Bisulfite-ozonated sea water rinse followed by a five minute soak in 1% bisulfite solution, ozonated ice storage.

Rinsing was accomplished by placing 2 pounds of shrimp in a stainless steel colander and pouring approximately 5 gallons of the appropriate rinse water over the shrimp. On board handling was simulated by shaking and swirling the colander. Four pounds of shrimp were used for each treatment. The treatments were placed in ice chests with approximately 8 pounds of ice and placed in refrigerated storage. Fresh ice of the appropriate treatment was added as required.

Shrimp from each treatment were tested on days 0, 2, 4, 6, 8, 11, 13, 15 and 18. On each test day the shrimp were analysed for aerobic plate count and evaluated for black spot and off odors. Aerobic plate counts were done by taking three samples of approximately 6 shrimp each and placing them in whirl pak bags. The shrimp were weighed and placed in waring blenders with appropriate amounts of 0.1% peptone dilution water and blended for 1 minute. Serial dilutions were plated on standard methods agar and incubated for 48 hours at room temperature. Black spot evaluations were made on the shrimp while they were still in the whirl pak bags. Off flavors were made while the shrimp were in the ice chests.

RESULTS AND DISCUSSION

Bacterial Analysis

The results of the bacterial analysis done in College Station are shown in Figure 1. The results for day 0 reflect the initial counts after deheading for the five treatments and clearly show the effect of rinsing on the initial bacterial load. There was a 56% reduction in initial counts due to rinsing the shrimp in a 1.25% bisulfite solution. The treatments that were not rinsed (ie., control, high ozone and low ozone) had initial counts of 8.7×10^4, whereas the bisulfite treated shrimp had initial counts of 3.8×10^4. The former treatments should have been rinsed in water as a control against the bisulfite rinse. This would have lowered the initial counts for these treatments and might have effected the overall analysis.

The results of the bacterial analysis done in Corpus Christi are shown in Figure 2. The results for day 0 reflect the initial counts after deheading and rinsing in the appropriate solution. The initial counts for this study, after deheading, were 1.6×10^4. Rinsing reduced bacterial numbers to 3.7×10^3, 2.4×10^3, 2.7×10^3 and 1.8×10^3 for control, fresh water, sea water and bisulfite treatments, respectively. This represents a 77% reduction for the control rinse, an 85% reduction for fresh water, an 83% reduction for sea water and an 89% reduction for the bisulfite rinse. This indicates the effect

of ozone in the rinses since the latter three rinses contained ozone. Although the control treatment was significantly higher than the others for day 0, no statistical analysis was done to determine whether these reductions were statistically different.

The difference in initial reductions between the two studies is due to the rinse methods used. In the College Station study, the shrimp were placed in a bucket containing the bisulfite solution and stirred for approximately one minute; whereas in the Corpus Christi study the shrimp were placed in a colander and the rinses were poured over the shrimp. The effect of the rinses in both studies was to extend the lag phase of the bacterial growth for 2-3 days. This apparently had no effect on the shelf life in either study, however it did effect the statistical analysis. The lower counts obtained in the initial part of the studies brought the overall averages down for these treatments and thereby affected the statistical comparisons between the overall means for each treatment.

Both studies were statistically analysed using analysis of variance and the treatment means were compared using the method of Scheffe (1953). Analysis of variance for both studies showed that there was a statistically significant effect due to the treatment methods. In the College Station study, the two bisulfite treatments had means that were significantly lower than the other three. There was no difference between the two bisulfite treatments and there was no difference between the other three (control, high ozone and low ozone). In the Corpus Christi study, there was no difference between the three treatments using ozonated ice, however the control was significantly higher than all three. This could be attributed to either the ozonated ice or the ozonated rinses.

By studying the graph in Figure 1, it is apparent that there was no effect due to storage on ozonated ice. The only effect in this study was due to the bisulfite rinse, which lowered the initial counts and delayed the lag period of bacterial growth. It can possibly be concluded that the amount of drip water could have effected the results (ie. delayed lag phase) for the bisulfite treatments since by day 5, 4600 ml of drip water was collected from these treatments compared to only 3600 for the other three. The drip water from the ice has a washing effect on the shrimp and can reduce the bacterial load. All treatments spoiled at the same rate when the bacteria reached the log or growth phase. At day 13, only the low ozone-bisulfite treatment was different. By day 17, all treatments were the same. Therefore it can be concluded that storage on ozonated ice had no effect on the bacterial spoilage of the shrimp used in this study (College Station).

Figure 2 shows that the ozonated ice treatments were lower than the control treatment for all days following day 7. This study (Corpus Christi) showed that storage of shrimp on ozonated ice could possibly have an effect on the bacteriological spoilage of shrimp, possibly 1-2 days extension of shelf life. The overall effect of the

ozonated rinses could be minimal because the effect did not show up until day 7. The difference between the two studies is that the Corpus Christi study used ice that was ozonated and made on the premises whereas in the College Station study the ice was made in Tyler and shipped to the lab in College Station. The ice used in the former study could have contained residual ozone where the ice used in College Station probably had lost its residual due to transportation and storage.

Chemical Analysis

Previous research, Bailey et al. (1956) and Vanderzant and Nickelson (1971) indicated that pH was a good indicator of shrimp spoilage (pH 8.0 was indicative of spoiled shrimp). The results in Figure 3 indicate the means for the ten observations taken for each treatment on each test day. Analysis of variance showed that there was no treatment effect on the pH of the shrimp throughout the study. However, on days 15 and 17 there was a significant difference between the control and the remainder of the treatments. This effect is cancelled due to the observations in the earlier part of the study. Therefore storage of shrimp in ozonated ice does not effect the pH of the shrimp. The results from the ammonia and total volatile nitrogen analysis are not presented due to errors in the analysis.

Distribution of Gram (-) versus Gram (+) Organisms

Table 1 summarizes the data obtained in this part of the study. The distribution of gram (-) organisms went from approximately 25% to approximately 95% for all treatments. This is in agreement with previous research reviewed by Nickelson and Vanderzant (1976) that showed that the bacterial flora shifted from mixed flora to predominately gram (-) flora under ice storage. Analysis of variance was not run on this data because the figures clearly show that there was no treatment effect due to the storage of shrimp on ozonated ice. Differences within the observations for certain days can be attributed to the number of organisms isolated for each treatment. The total number of isolates for a particular treatment are solely dependant on the aerobic plate counts for that treatment.

Black Spot Analysis

Due to the fact that ozone is a strong oxidizing agent, there was a concern that ozone would either accelerate the oxidation of tyrosine or oxidize bisulfite, thereby accelerating the formation of blackspot. The results of the black spot evaluation in the College Station study are summarized in Table 2. The Corpus Christi evaluations are found in Table 3. In the College Station study, there was no black spot noted until day 5 of the study. The only treatment effect that was seen was due to the bisulfite rinse and not the ozonated ice storage. The highest incidence of black spot was found on the ozone stored shrimp on day 15. In the Corpus Christi study, black spot was found on day 2 of the study. The incidence of black was higher in these shrimp than in the College Station study. In this

phase the bisulfite rinsed shrimp were also the lowest in percent black spot. Therefore ozone had no effect on the formation of blackspot in shrimp stored on ozonated ice.

CONCLUSIONS

In the College Station phase of this study, the use of ozonated ice had no effect on the shelf life of shrimp stored on ice. In the Corpus Christi phase of this study, ozonated ice was shown to possibly prolong the shelf life of shrimp stored on ice for 1-2 days. However, this study was unable to distinguish whether the extension of shelf life was due to the use of ozonated rinses or to the use of ozonated ice for storage. The major problem was whether the ice contained any residual ozone. In the College Station study, the ice probably did not contain ozone, but in the Corpus Christi phase, there may have been ozone in the ice since the effect of the ozone did not appear until day 7 of the study. The use of ozonated ice had no effect on the incidence of black spot in either study. In future studies using ozonated ice, some method of determining the residual ozone concentration in the ice should be utilized. This was not done in this study, however it would have clarified the confusion as to whether the ice or the rinse was beneficial in prolonging the shelf life of the shrimp.

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Figure 1. Graph of log bacteria count versus days in storage for first phase of study (College Station).

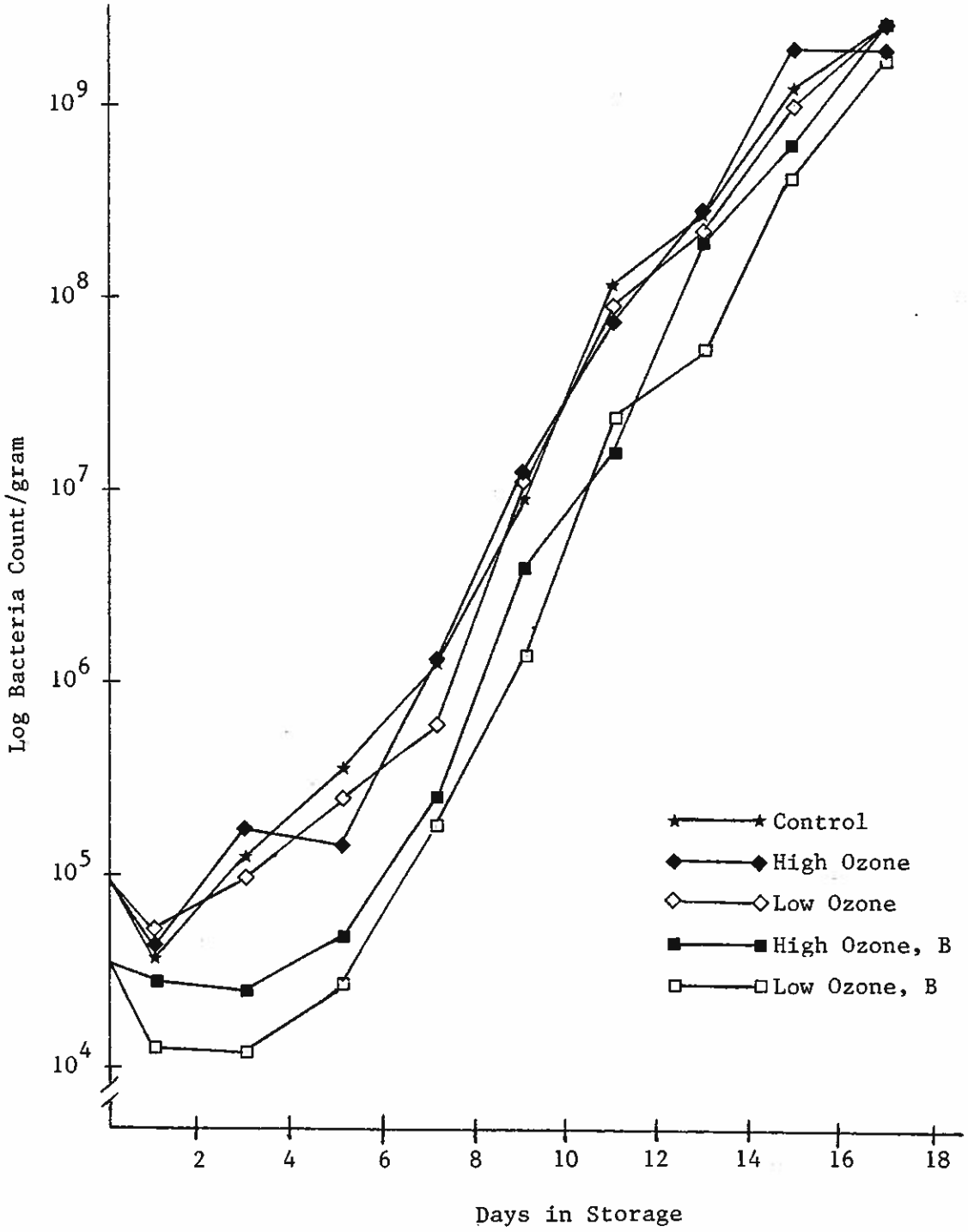

Figure 2. Graph of log bacteria count versus days in storage for second phase of study (Corpus Christi).

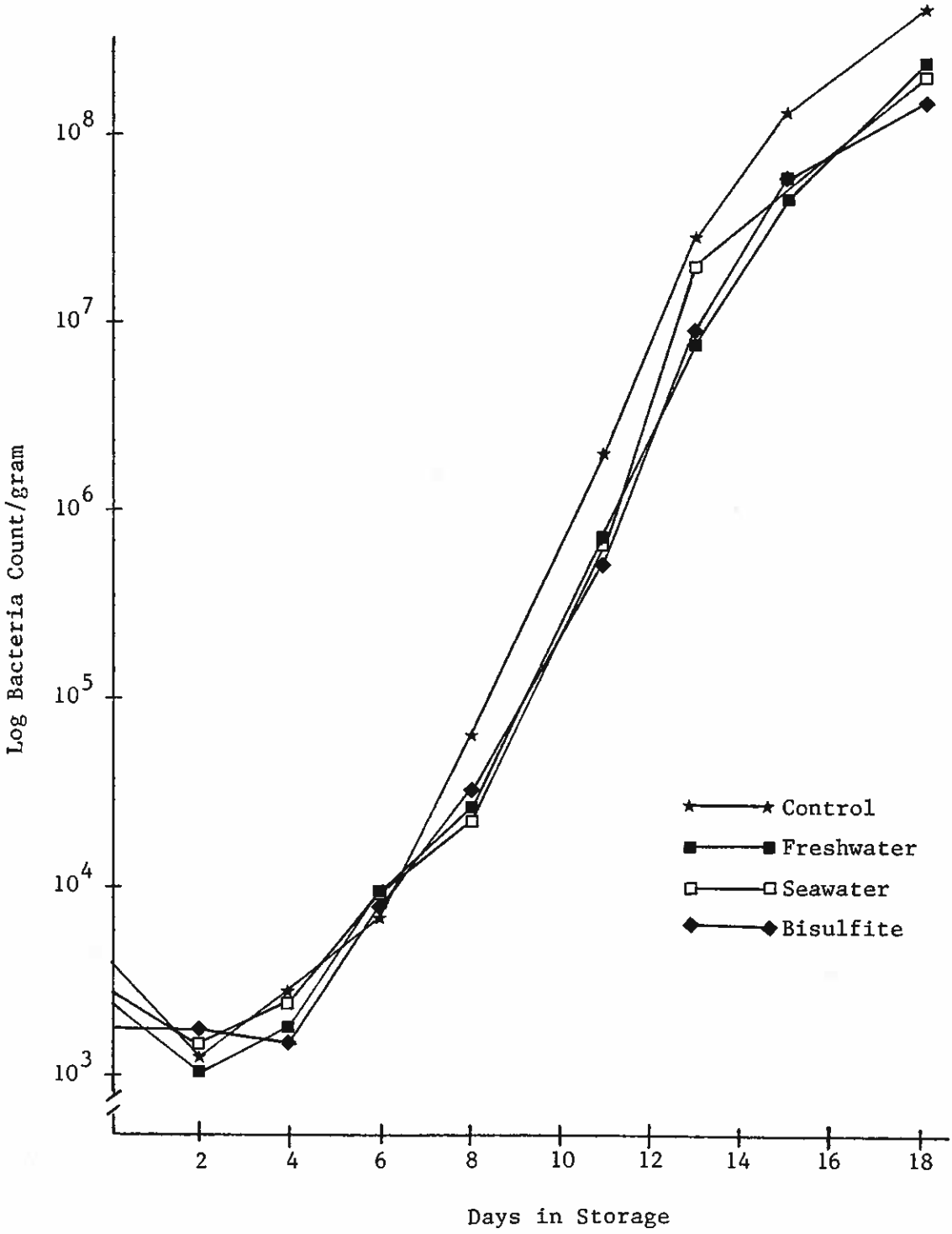

Figure 3. Graph of pH versus days in storage for first phase of study (College Station).

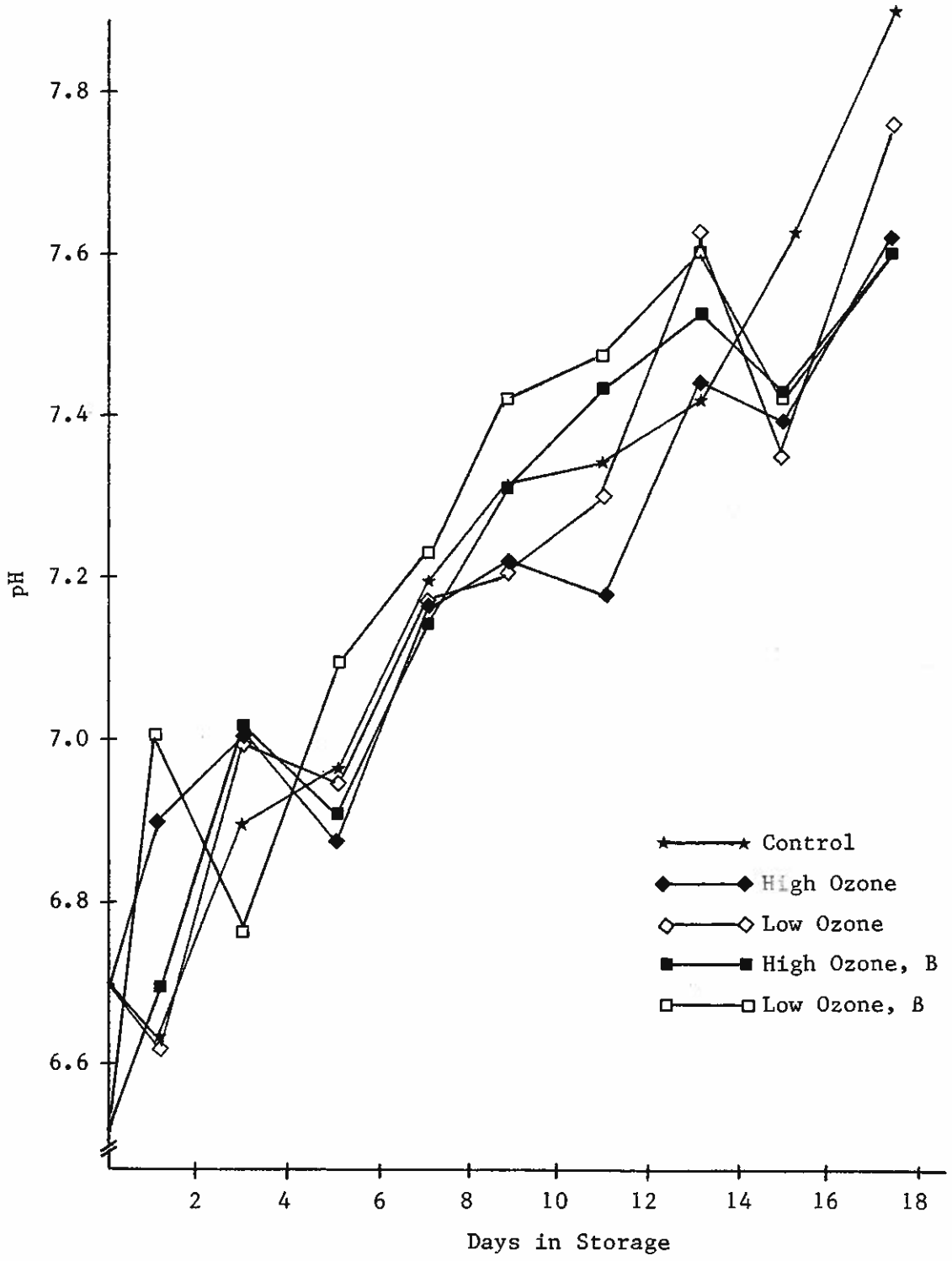

Table 1. Distribution of gram (+) versus gram (-) organisms from College Station study.

DAY	CONTROL		OZONE (H)		OZONE (L)		OZONE (H) BISULFITE		OZONE (L) BISULFITE	
	% G+	% G-	% G+	% G-	% G+	% G-	% G+	% G-	% G+	% G-
0	75.0	25.0	75.0	25.0	75.0	25.0	77.0	23.0	77.0	23.0
1	73.5	26.5	76.0	24.0	77.5	22.5	82.5	17.5	78.0	22.0
3	74.5	25.4	69.5	30.5	75.5	24.5	61.5	38.5	51.0	49.0
5	77.0	23.0	65.0	35.0	68.0	32.0	42.7	57.5	58.5	41.5
7	35.5	64.5	45.5	54.5	49.0	51.0	45.0	55.0	61.0	39.0
9	39.0	61.0	20.0	80.0	30.5	69.5	24.5	75.5	26.5	73.5
11	12.0	88.0	37.0	63.0	10.5	89.5	22.5	77.5	12.0	88.0
13	11.0	89.0	19.5	80.5	9.0	91.0	13.0	87.0	14.0	86.0
15	8.5	91.5	7.5	92.5	8.5	91.5	10.0	90.0	7.0	93.0
17	6.5	93.5	5.5	94.5	6.5	93.5	3.5	96.5	4.5	95.5

Table 2. Results of black spot analysis (%) done in College Station.

DAY	CONTROL	OZONE (H)	OZONE (L)	OZONE (H) BISULFITE	OZONE (L) BISULFITE
0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0
5	0.0	12.0	24.0	8.0	0.0
7	40.0	20.0	12.0	0.0	0.0
9	52.0	40.0	12.0	8.0	12.0
11	52.0	44.0	32.0	32.0	8.0
13	60.0	52.0	48.0	12.0	24.0
15	52.0	76.0	72.0	52.0	24.0
17	40.0	44.0	44.0	24.0	28.0

Table 3. Results of black spot analysis (%) done in Corpus Christi.

DAY	CONTROL	FRESH WATER	SEA WATER	BISULFITE
0	0.0	0.0	0.0	0.0
2	30.0	33.0	30.0	5.0
4	24.0	33.0	29.0	10.0
6	28.0	49.0	33.0	11.0
8	38.0	33.0	44.0	11.0
11	49.0	77.0	71.0	44.0
13	66.0	77.0	70.0	60.0
15	82.0	65.0	93.0	55.0
18	71.0	82.0	93.0	88.0

EVALUACION DE LA CALIDAD PROTEINICA DE LA HARINA DE CARNE DE TIBURON TOLLO (Squalus acanthias)

Armando lacera Rua, Ricardo Bresssani,
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El presente trabajo informa sobre la calidad proteínica de la harina de tiburón tollo evaluada oir varios métodos biológicos, incluyendo diges tibilidad. El producto contenía 91.52% de proteína cruda total y 69.86% de proteína verdadera, siendo la diferencia nitrógeno no proteico, princi palmente urea. Por esta última razón, las evaluaciones biológicas con los métodos usados se realizaron con diferentes concentraciones de harina de tiburón, evaluados en diferentes tiempos para cada método. Se prepararon dietas con 0, 3, 6, 9, 12% de harina de tiburón evaluadas por el método de PER, NPR y NGI. Se utilizó caseína como proteína de referencia. La dife s tibilidad de la proteína fue de 91.2% sin corrección por el nitrógeno no proteico. En estas mismas bases, el PER y NPR a un nivel de proteína de 9.97% en la dieta fueron de 2.58 y 2.05, respectivamente. El control de caseína dio un PER de 2.93 y un NPR de 4.52. El BGI fue calculado usando la dieta aproteica o sin ella, con valores de 3.32 y 2.95, respectivamente. Todos los valores corregidos por el nitrógeno no proteico fueron mayo res, como era de esperarse, excepto la digestibilidad de la proteína. Las dierencias fueron significativas al 5%. Aparentemente, la urea no tuvo - una influencia negativa, sobre las evaluaciones biológicas. Se concluye, por consiguiente, que la carne de tiburón es de excelente calidad.

INTRODUCCION

En años recientes se han realizado considerables esfuerzos para incre mentar la utilización de los recursos de los oceános para la alimentación humana. Se ha hecho hincapié en la utilización completo de los productos marinos como nuevas fuentes de proteínas, tales como las especies de peces no convencionales (1).

El tiburón es una fuente potencial de proteínas para el consumo inter no en los países productores; sin embargo, se trata de un recurso que con frecuencia no se utiliza plenamente o incluso se desperdicia. En comparación con los otros peces, la carne de tiburón es magra y un poco ácida. El contenido porcentual de proteína es diferente según las distintas especies, pero corresponde al de varias especies de teleósteos en lo tocante a la composición y distribución de aminoácidos esenciales. El contenido de lisina, que es de especial importancia en la harina de pescado, es el mismo en la carne de los tiburones que en la de los demás peces (2).

Kizevetter y Nasedkina (3) aseguran que la concentración de los amino ácidos esenciales en la proteína del tiburón tiende a ser menor con respec to a la observada en la carne de res y que, además, las deficiencias relati vas dependen de la especies, por lo cual estos autores colocan la carne de los elasmobranquios como de bajo valor alimenticio.

Este trabajo tuvo los siguientes objetivos: a) evaluar la calidad de la proteína de la carne del tiburón tollo (Squalus acanthias), de distribu ción comercial para consumo directo en Guatemala; b) Revisar la información disponible sobre la calidad proteínica de harinas de otras especies de pes cados; c) comparar estos datos con los obtenidos en el presente estudio tratar de explicar las razones por las cuales la harina de tiburón

tollo no presenta las restricciones nutricionales mencionadas en la litera tura, en relación con otras harinas de peces teleósteos y demas carnes (3).

MATERIALES Y METODOS

Harina de carne de tiburón desecada

El tiburón tollo fue adquirido en el mercado local. Los trozos de car ne fresca se molieron en un molino eléctrico de discos, para luego colocar los en bandejas de tela metálica en un deshidratador de aire caliente por un período de 16 horas, con una temperatura de aire entrante de 60°C. Una vez seca, la carne fue triturada para preparar un material de granulometría uniforme.

El material así preparado fue sometido a un análisis proximal de acuer do con los métodos de la AOAC (4) y el nitrógeno no proteíco se determinó - después de precipitar la proteína con ácido tricloracético al 5% (4). El extracto libre de nitrógeno se estimó por diferencia.

Dietas

Con el material seco se prepararon 4 dietas con 3, 6, 9 y 12% de pro- teína de la carne de tiburón. A todas las dietas se les adicionó en g por cada 100 gramos: 5 celulosa, 1 de aceite de hígado de bacalao, 5 de aceite vegetal refinado, 4 de mezcla mineral (5,6) y 5 ml de una solución de vita mina del complejo B. Las dietas se llevaron a 100% con almidón de maíz (5) y fueron analizadas por nitrógeno por el método de Kjeldahl (4), y las ca- lorías calculadas por los factores de Atwater (7). Como control se utili- zó caseína al 9%, así como también una dieta libre de nitrógeno.

Ensayos biológicos

Se utilizó un total de 48 ratas Wistar de 21 días de edad distribuidas por peso entre las dietas experimentales, 8 por grupo, 4 hembras y 4 machos. Los animales fueron colocados en jaulas individuales con fondos de tela me tálica elevados. Se les administró la comida y el agua ad líbitum anotando los cambios de peso y consumo semanalmente. La calidad proteíca de la hari na de carne de tiburón fue evaluada por medio de tres parámetros biológicos: el PER (Razón de Eficiencia Proteínica), el NPR (Razón Neta Proteínica) y el NGI (Indice de Crecimiento Nitrogenado). Para los ensayos de NPR y NGI se usó una dieta aproteica, de igual composición que la descrita, sustituyendo la cantidad de harina de pescado por un peso igual de almidón de maíz. Al finalizar los 28 días de la prueba del PER, se les administró a los animales 60 g de la dieta correspondiente, y durante 3 días se recolectaron las mate rias fecales. Estas fueron secadas en horno de aire forzado a 60°C durante 10 horas, previo a su análisis de nitrógeno para la determinación de la Di- gestibilidad Aparente.

RESULTADOS Y DISCUSION

Análisis proximal

En la Tabla 1 se presentan los valores porcentuales de nitrógeno (total y no proteíco), urea (referida al nitrógeno total, proteína cruda (N x 6.25, referida tanto al nitrógeno total como a la diferencia de éste con el proteíco), extracto libre de nitrógeno (por diferencia), para la harina de carne de tiburón y para cada una de las dietas.

Se nota que el nitrógeno no proteíco (NNP) representa una cifra importante del contenido total de nitrógeno. El NNP representa un promedio de 23.7%, con respecto alnitrógeno total.

Los porcentajes de urea en las dietas comprenden un rango (de nitrógeno no proteíco) entre 25.2 y 32% del nitrógeno total. Debido a estos niveles relativamente altos de NNP, el producto bajo estudio se evaluó por diferentes métodos biológicos, usando dietas con diferentes niveles de proteina y pruebas de diferentes tiempos de duración.

Es importante relacionar los valores de NNP y de urea con los encontrados por Kizevetter y NaSEDKINA (3), quienes informaron que del contenido total de nitrógeno, solamente el 50-64% representa nitrógeno proteíco y el 50-36% nitrógeno no proteico, cuya fracción porcentual principal es nitrógeno ureico.

Calidad proteínica

En la Tabla 2 se presentan los resultados encontrados para los PER de las diferentes dietas. Cabe indicar que no hubo diferencias significativas entre las respuestas encontradas en machos y hembras (al nivel del 5%), lo cual puede deberse a que los grupos fueron compuestos del mismo número de animales de cada sexo.

El PER se calculó tanto en función del nitrógeno total como corrigiendo éste por elnitrógeno no proteico. Los valores más altos se encontraron cuando se corrigió el nitrógeno total por el NNP, aunque en los dos tipos de cálculo los resultados mostraron la misma tendencia.

El PER promedio de machos y hembras para la dieta de nivel 9 (proteína 9.97%) fue de 2.58 $\pm$ 0.16. El control de caseína para el presente ensayo fue de 2.93 $\pm$ 0.12.

Séve, Aumaitre y Tord (8) han obtenido un PER en ratas de 2.83 para harina de pescado de músculo blanco prensada y desgrasada parcialmente; la harina fue obtenida de los desechos de fileteados.

Bourges, Gaona y Haas (9) determinaron el PER (en ratas) de tortas de pescado almacenado durante 18 meses usando un nivel de proteína cruda del

10%; el valor encontrado fue de 2.8 ± 0.4. Islam y Lear (10) encontraron un PER de 4.23 ± 0.25 alnivel del 9% en concentrado proteico de Mummichog (Fundulus heteroclitus) y un valor de 3.36 para el control de caseína.

Bonsembiante, Susmel y Cesselli (11) obtuvieron un PER (en ratas) de 2.53 para harina de arenque, determinando valores comparativos de PER para harina de soya y el control de caseína de 2.20 y 2.72 , respectivamente.

Fernández Otero et al.(12) alimentaron ratas recién destetadas (raza Wistar) con carne fresca de lamprea (Petomyzon marinus) al nivel del 12% utilizando caseína como control. Los PER encontrados fueron: 2.38 para la carne de lamprea y 2.73 para la caseína, respectivamente.

Pronczuk et al. (13) estudiaron el valor nutritivo de harinas y concentrados proteicos de pescado y encontraron que a un nivel de 10% de proteina los PER fueron de 2.64 y 2.46, respectivamente.

Morrison et at. (14) encontraron un PER de 2.59 en harina de arenque sin espina, utilizando un nivel de proteína del 10%. El control de caseína produjo un PER igual a 2.5.

Los resultados obtenidos con la dieta de nivel 9 del presente estudio son comparables con los anteriores, es decir que la harina de tiburón sigue un comportamiento de respuesta, en relación con el PER, similar al de los peces teleósteos.

Razón de Eficiencia Proteínica (PER) corrigiendo el nitrógeno total por nitrógeno no proteíco. En la Tabla 2 se resumen los resultados y se observa que los PER calculados de esta forma son mayores que los obtenidos sin corregir por nitrógeno no proteíco; esto es de esperarse, ya que para el mismo aumento en el peso hubo una mejor ingestión de proteína verdadera.

Las diferencias se plantean en una base aclaratoria, ya que el tiburón como elasmobranquio contiene urea y es posible que su nitrógeno, así como el nitrógeno no proteico de otros compuestos nitrogenados, tengan influencia en la respuesta de PER y demás determinaciones biológicas del presente trabajo. Es importante aclarar que parte delnitrógeno no proteico de la leche humana está en forma de urea (15).

Se ha discriminado en nitrógeno total y nitrógeno total corregido por nitrógeno no proteico, con el fin de observar la influencia de este último en los resultados. No es aconsejable la diferenciación entre las dos categorías anteriores de nitrógeno, debido a que el nitrógeno de aminoácidos libre puede ser del mismo valor nutricional que el de la proteína (5) pero por otro lado, no está clara aún la significancia nutricional de algunos nitrógenos no aminoacídicos y no peptídicos, y es así como se considera usualmente el análisis nitrogenado del alimento mucho más preciso que su significancia nutricional (5), por lo cual hay que considerar el cálculo de cada

valor de PER, NPR, NIG y digestibilidad aparente del presente ensayo, la influencia del nitrógeno ureico y demás nitrógeno no proteico en cada dieta particular; sin embargo, para propósitos prácticos de comparación, deben tenerse en cuenta sólo las respuestas obtenidas con "nitrógeno total no corregido por nitrógeno no proteico".

Razón Proteínica Neta (NPR). En la Tabla 2 se resumen los valores promedios de NPR. No hubo diferencias significativas entre machos y hembras al nivel de 0.05, por la prueba "t" de Student.

La Tabla 2 también resumen los valores de NGI, estimados por regresión linear a los 14 días de ensayo, usando, en un caso, el valor de la dieta aproteica y, no usando, en el otro, dicho valor. Semejante al PER y al NPR, los valores fueron calculados usando el nitrógeno total y éste corregido por el nitrógeno no proteico. El coeficiente de regresión es equivalente al valor proteínico del producto. Así como para el PER y el NPR, los valores calculados con base en el nitrógeno total corregido por el NNP son mayores, como es de esperarse.

Digestibilidad aparente. En la Tabla 2 se presentan los resultados calculados con la dieta de nivel 9, considerando nitrógeno total y nitrógeno total corregido por nitrógeno no proteico.

Se encontraron valores promedio de digestibilidades aparentes, idénticos en machos y hembras (91.18 $\pm$ 1.58 y 91.18 $\pm$ 1.51, respectivamente), calculados por nitrógeno total; en el cálculo con nitrógeno corregido por NNP, los valores promedio de machos también fueron idénticos a los de hembras (88.44 $\pm$ 2.07 y 88.44 $\pm$ 1.98, respectivamente). Como se observa, estos últimos valores de digestibilidades son menores que en el caso de considerar el nitrógeno total como proteico. El análisis por "t" de Student demostró total como proteico. El análisis por "t" de Student demostró que no hay ninguna diferencia significativa alnivel del 5%, entre machos y hembras, como se expresa en una u otra forma.

Zombade y Sathe (16) encontraron valores de digestibilidad aparente en un rango de 52 a 71% en músculo de diez especies de pescados, utilizando pepsina.

Séve, Aumaitre y Tord (8), encontraron una digestibilidad aparente de 90% en harina preparada con desechos de fileteado y proteolizada enzimáticamente; la misma harina desgrasada por doble centrifugación dio un valor de digestibilidad aparente de 91.4%. En ambos casos, el nivel de proteína fue del 10%.

Pronczuk et al. (13), estudiando el valor nutritivo de algunas harinas de pescado y concentrados de pescado, encontraron, en ratas, una digestibilidad aparente de 75.9 y 72.9%, respectivamente, y al 10% de proteína.

Tsuladze y Giazaryan (17) estudiaron la digestibilidad aparente de músculos de pescado, cercanos a la cabeza, Los músculos fueron cocidos en H_2O a 75° C.por 30 min. Las digestibilidades se estimaron en solución de pepsina cristalina a 37°C por 3 horas, seguido de un tratamiento similar con solución de tripsina cristalina. Las anteriores soluciones de enzimas fueron

preparadas en forma similar a predominantes en el jugo gástrico o pacreáti co humanos. Los resultados encontrados por estos autores se presentan en la Tabla 3.

Los valores de digestibilidad para pescados señalados anteriormente en la literatura son del mismo orden que los encontrados en el presente traba jo en ratas, la proteína de la harina de tiburón.

Es interesante mencionar cómo sólo los valores de digestibilidad apa- rente con la mezcla enzimática pepsina + tripsina son un poco mayores que la digestibilidad de la carne de tiburón (93.3) (White Amur) y 98.3% (Cabe zón).

Análisis gráfico

Variación de PER con respecto al nivel de proteína. En las figuras 1 y 2 se presentan la variación del PER versus nivel de proteína, consideran do nitrógeno total y nitrógeno corregido por no proteico, respectivamente.

En ambos casos se observa que los máximos corresponden al promedio de ratas hembras. Es claro que al incrementarse el nivel de proteína hubo me nor variabilidad (menor desviación estándar) en las respuestas de PER. Con excepción del nivel del 12%, las ratas hembras, en promedio, mostraron una tendencia general menor de desviación estándar con relación a las ratas ma chos.

Variación de PER con el tiempo. Los datos al respecto se presentan en la figura 3. El comportamiento de las curvas es muy similar al encontra do por Bressani et al. (18) para harinas de girasol, de soya, de maní y de algodón.

Las ratas hembras presentaron una caída un poco mayor en el PER con el tiempo, en comparación a las ratas machos. También aquí sólo se tuvo en cuenta la dieta con 9.97% de proteína.

Es importante mencionar cómo en las variaciones de PER y NPR con el nivel de proteína, los máximos valores respectivos se alcanzaron con la - dieta con 6% de harina de tiburón, a la cual le correspondió un 0.240% de urea. Surge el interrogante de si a este nivel de proteína las ratas uti lizaron o no ese porcentaje de urea para su crecimiento. Estudios anterio res sugieren la utilización del nitrógeno de urea; Tylecek, Skalova y Zed nik (19) encontraron que ratas y cerdos alimentados con cereales con un bajo nivel de nitrógeno y con suplementos de urea al 1% (de las dietas) - tuvieron ganancia positiva de peso y que, además, el suplemento al 1% tuvo un efecto positivo mayor que el suplemento al 2.5% (19).

Por otro lado, Erickson et al. (15) hace cinco décadas comentaron que los compuestos nitrogenados no proteicos de la leche podrían tener alguna influencia en la nutrición del infante.

Resalta la importancia de realizar otros ensayos de carne de tiburón tollo en relación con otras especies de pescado, a fin de cuantificar el efecto nutricional, positivo o negativo, de la urea contenida en dicho eslas mobranquio.

Variación del NPR con el nivel de proteína. Estos dos aspectos se en cuentran relacionados en la figura 4. Los NPR fueron calculados a los 14 días y con nitrógeno total en cada una de las dietas.

las formas de las curvas fueron similares a la encontrada entre la variación de PER versus nivel de proteína de las dietas, excepto que las ratas machos no siguieron en la curva la tendencia uniforme de "una curva sobre otra".

Variación del NPR con respecto al tiempo. En la figura 5 se nota cla ramente la forma en que decreió el NPR al transcurrir el tiempo. Las ra - tas hembras presentaron la mayor desviación estándar en un intervalo de - tiempo determinado.

Para propósitos comparativos, se utilizaron sólo los NPR calculados con nitrógeno total correspondientes a 9.97% de proteína; se alcanzaron mayores NPR promedios en machos y en hembras a los siete días.

Indice de Crecimiento Nitrogenado, NGI_o. En las Figuras 6 y 7 se han trazado las rectas de regresión calculadas al relacionar la variación de peso con el consumo de proteína. Se han considerado los valores obteni dos a partir de la dieta libre de nitrógeno. La Figura 6 corresponde a valores calculados con nitrógeno total y la figura 7 a los del nitrógeno corregido por NNP.

En la figura 6, la mayor pendiente (mayor NGI_o) se obtuvo a los 7 días (3.79); luego decreció a los 14 días (3.32), y aún más a los 21 días (2.96). Por otro lado, los coeficientes de correlación fueron altos, entre 0.96 y 0.97.

En la figura 7 se nota un decrecimiento del NGI_o con relación al tiem po, pero los coeficientes de correlación fueron ligeramente menores en com paración al de la Figura 6.

Similar al PER y al NPR, los NGI_o (considerando NNP) son mayores que los NGI_o (considerando/nitrogenado total), en igual intervalo de tiempo. En ambos casos no hubo diferencias significativas entre machos y hembras al nivel del 5%.

Wolzak (20) encontró un NGI_o igual a 3.98 con intercepto de -1.02 para caseína; en harina de algodón + 0.3% L-lisina encontró un NGI de 3.12 e intercepto de -1.01; en el caso de harina de soya, el valor de NGI_o encontrado fue de 3.74 con un intercepto de -0.899. Los valores de NGI_o encontrados por Wolzak en creales son menores que el NGI_o en la carne de tiburón: maíz (2.37), trigo (2.42), triticale (2.61), con excepción del arroz, que tuvo un valor mayor (4.18). Dichos ensayos fueron realizados al nivel de - 10% de proteína, con excepción del arroz (6) y maíz (8%).

Al utilizar el criterio de considerar el intercepto en X (gramos de proteina) cuando Y = 0 (variación de peso), como indicación de cantidad mínima de proteína evaluada necesaria para mantenimiento, se obtiene un valor de 0.413 g de proteína de tiburón, aproximadamente la mitad del calculado para caseína (0.850)

NGI (según Hegsted). En las figuras 8 y 9 se han trazado las rectas de regresión obtenidas de igual forma que en el NGI, pero en los cálculos no se han incluido los valores de la dieta libre de nitrógeno (DLN).

La variación del NGI fue similar a la del caso anterior; disminuyó al transcurrir eltiempo. Los coeficientes de correlación fueron menores en relación con los de NGI_o (0.84, 0.92, 0.93 respectivamente).

Por otro lado, los presentes NGI son menores si se relacionan, en igual intervalo de tiempo, con los NGI_o (3.63 vrs. 3,79; 2.95 vrs. 3,32; y 2.57 vrs. 2.96).

Idéntico comportamiento se obtuvo en la figura 9, donde se consideró el nitrógeno total corregido por NNP. Similarmente, el caso de NGI_o calculados con nitrógeno corregido por NNP fueron mayores que los NGI obtenidos con nitrógeno total (Tabla 1).

SUMMARY

EVALUATION OF THE PROTEIN QUALITY OF TOLLO SHARK (Squalus acanthias) MEAT FLOUR

Rhe present study reports the protein quality of tollo flour evaluated by various biological methods, including digestibility. The product containd 91.52% crude protein and 69.86% true protein, the difference being non-protein nitrogen, mainly urea. Due to this reason the biological evaluations were carried out with different shark flour concentrations and evaluated at different lengths of time for each method. Diets with 0, 3, 6, 9 and 12% shark flour were prepared and evaluated by the PER, NPR and NGI methods. Casein was utilized as reference protein. The protein digestibility was 91.2%, not corrected for non-protein nitrogen. Under this sa

me basis, PER and NPR at a protein level of 9.97% in the diet were 2.58 and 2.05, respectively. The casein control diet gave a PER of 2.93 and an NPR of 4.52. NGI was calculated using the non-protein diet or without it, with values of 3.32 and 2.95 respectively. All values corrected for non-protein nitrogen were bigher, as was expected, except at 5%.

Aparently, urea influenced negatively the biological evaluations. It can be concluced, therefore, that the tollo shark meat is of excellent quali ty.

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TABLA 1

COMPOSICION PROXIMAL DE LAS DIETAS ELABORADAS CON HARINA DE TIBURON TOLLO (Squalus acanthias)

Harina de tiburón (Nivel en la dieta)	Humedad	Ceniza	Grasa	Nitrogeno	NNP	Urea (referida a nitróge no total)	Proteina p^a x 6.25	Proteina p^b	ELN*	Cal/g**
0	14.23	4.17	13.88	0	0	0	0	0	67.72	3.96
3	14.21	3.56	13.89	0.51	0.12	0.13	3.19	2.44	60.15	3.78
6	13.54	3.92	13.54	1.10	0.26	0.24	6.89	5.26	75.11	3.78
9	14.57	3.97	8.78	1.60	0.38	0.34	9.97	7.61	57.71	3.50
12	12.15	4.25	13.40	2.08	0.49	0.56	12.99	9.91	50.21	3.73
Harina integral de tiburón	3.98	2.1	2.4	14.64	3.47	3.55	91.52	69.86	-	3.88

* Por diferencia (extracto libre de nitrógeno)

** Por factores de Atwater.

p^a = Nitrógeno total x 6.25.

p^b = (Nitrógeno total - NNP) x 6.25.

Todas las dietas contienen 5% de fibra celulosa.

TABLA 2

CALIDAD PROTEINICA DE LA HARINA DE TIBURON TOLLO[a]

	1[b]	a[c]	Caseína[d]
PER	2.58 ± 0.16	3.37 ± 0.21	2.93 ± 0.12
NPR	2.05 ± 0.42	2.71 ± 0.57	4.50 ± 0.54
NGI_o[e]	3.32 ± 0.96	4.37 ± 0.88	4.51[f]
NGI[g]	2.95 ± 0.92	3.77 ± 0.97	-
Digestibilidad aparente, %	91.2 ± 1.4	88.4 ± 1.9	-

[a]9.97 de proteina.

[b]Calculado con el nitrógeno total de la dieta.

[c]calculado con el nitrógeno total de la dieta corregida por el nitrógeno no proteico.

[d]9.0% de proteina

[e]Tomando en consideración la dieta aproteica (14 días); $Y = a \pm bX$.

[f]Wolzak (2).

[g]Excluyendo el valor de la dieta aproteica (14 días); $Y = a \pm bX$.

TABLA 3

DIGESTIBILIDAD APARENTE POR METODOS ENZIMATICOS EN PESCADO

Pescado	Digestibilidad aparente, %	
	Pepsina	Pepsina-tripsina
White Amur (Ctenopharyngodon Idella)	85.3	93.3
Cabezón (Aristichthys nobilis)	88.7	98.3
Turbot (Thombus maloticus)	83.0	88.5
Grey Mullet (mugil auratus)	80.3	86.5

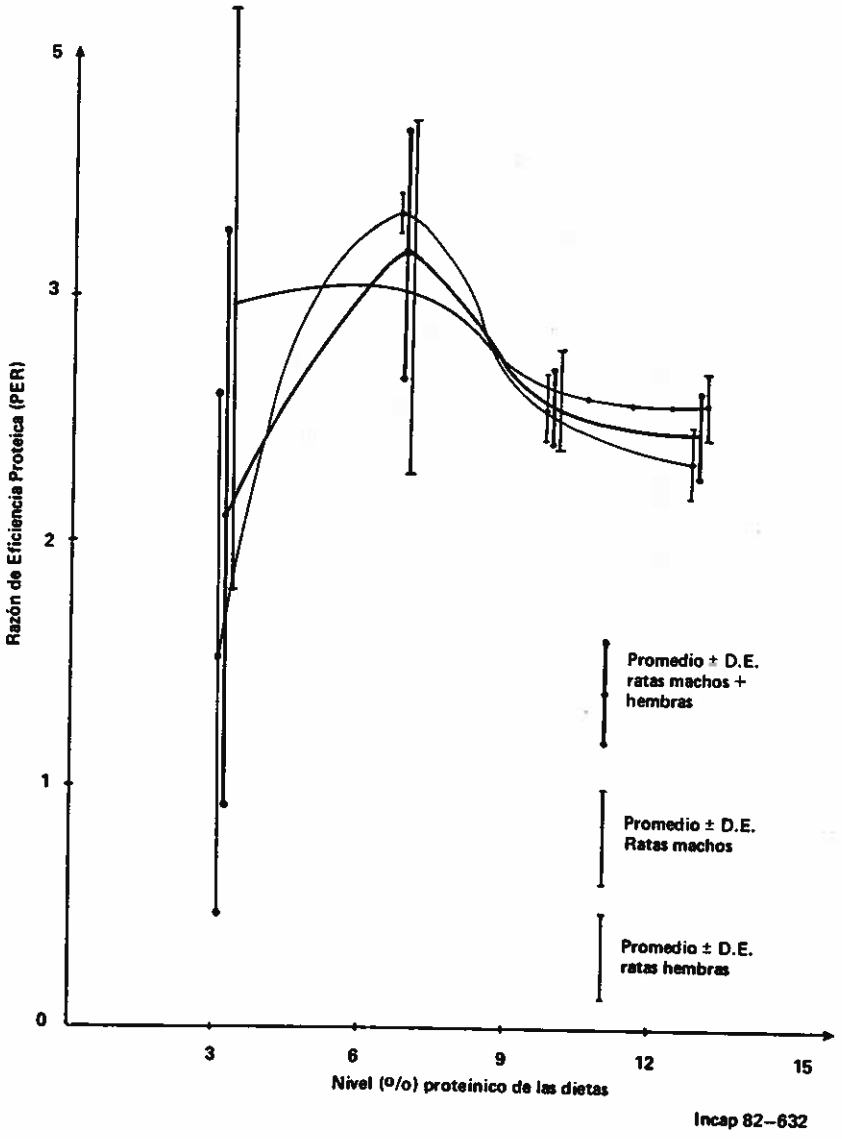
5
3
2
1
0
Razón de Eficiencia Proteica (PER)
3
6
9
12
15
Nivel (%) proteínico de las dietas
Promedio ± D.E.
ratas machos +
hembras
Promedio ± D.E.
Ratas machos
Promedio ± D.E.
ratas hembras
Incap 82-632

FIGURA 1

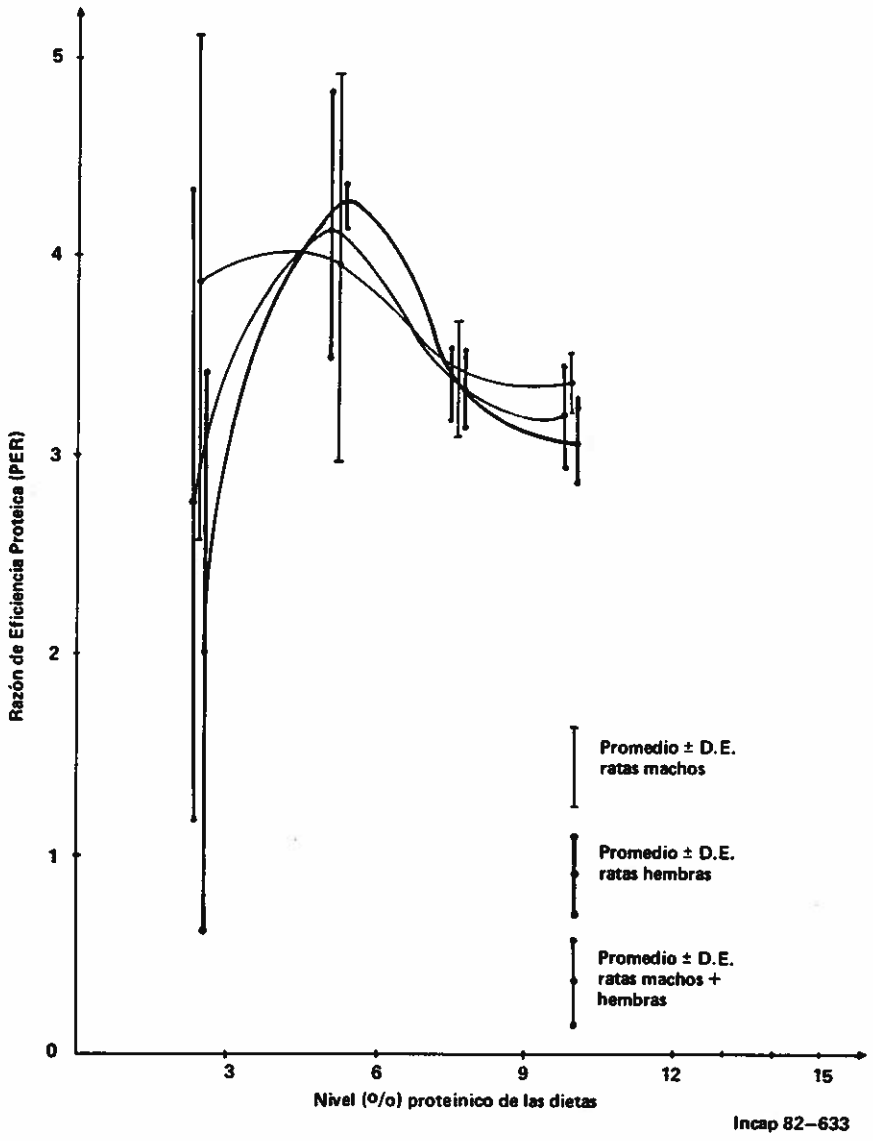
Razón de Eficiencia Proteica (PER)
5
4
3
2
1
0
3
6
9
12
15
Nivel (o/o) proteinico de las dietas
Promedio ± D.E.
ratas machos
Promedio ± D.E.
ratas hembras
Promedio ± D.E.
ratas machos +
hembras
Incap 82-633

FIGURA 2

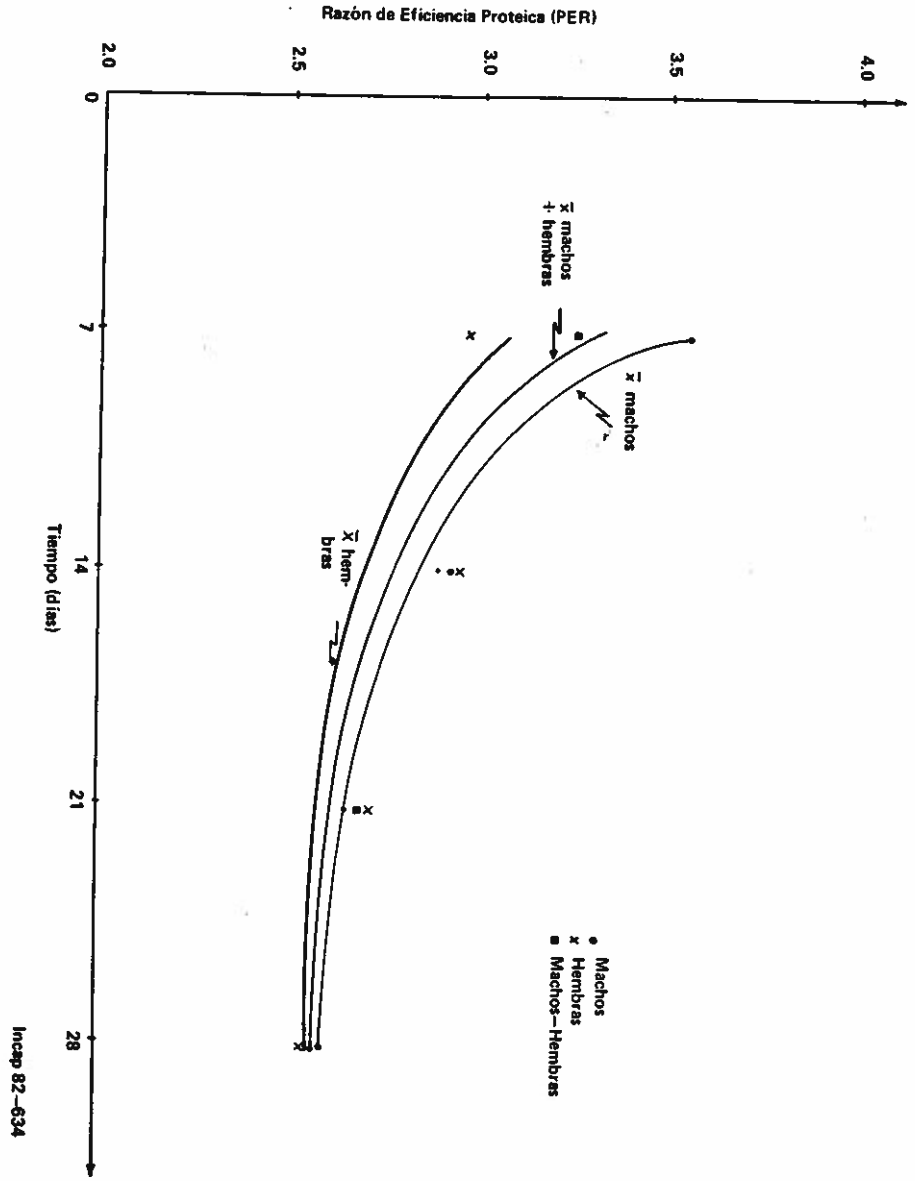
Razón de Eficiencia Proteica (PER)
2.0
2.5
3.0
3.5
4.0
0
7
14
21
28
Tiempo (días)
x̄ machos + hembras
x̄ machos
x̄ hembras
Machos
Hembras
Machos–Hembras
Incap 82–634

FIGURA 3

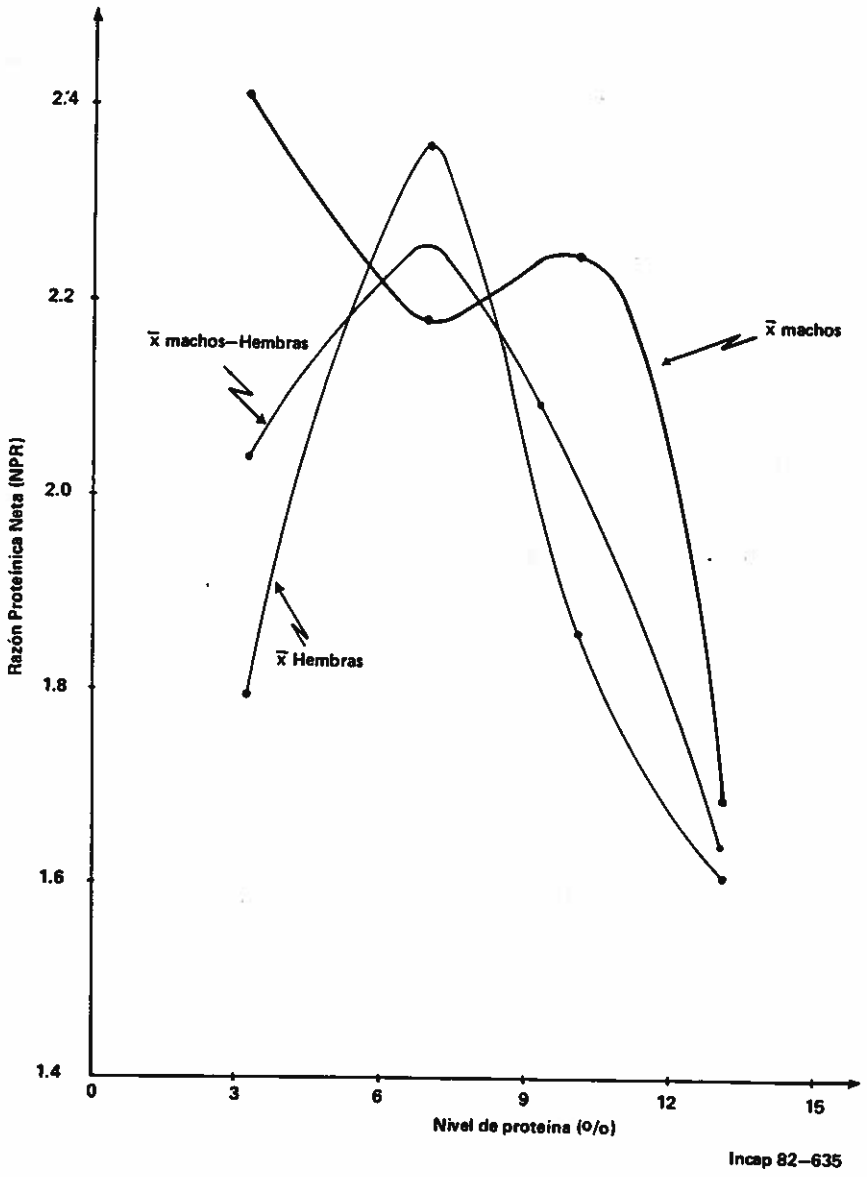
2.4
2.2
2.0
1.8
1.6
1.4
Razón Proteínica Neta (NPR)
x̄ machos–Hembras
x̄ machos
x̄ Hembras
0
3
6
9
12
15
Nivel de proteína (o/o)
Incap 82–635

FIGURA 4

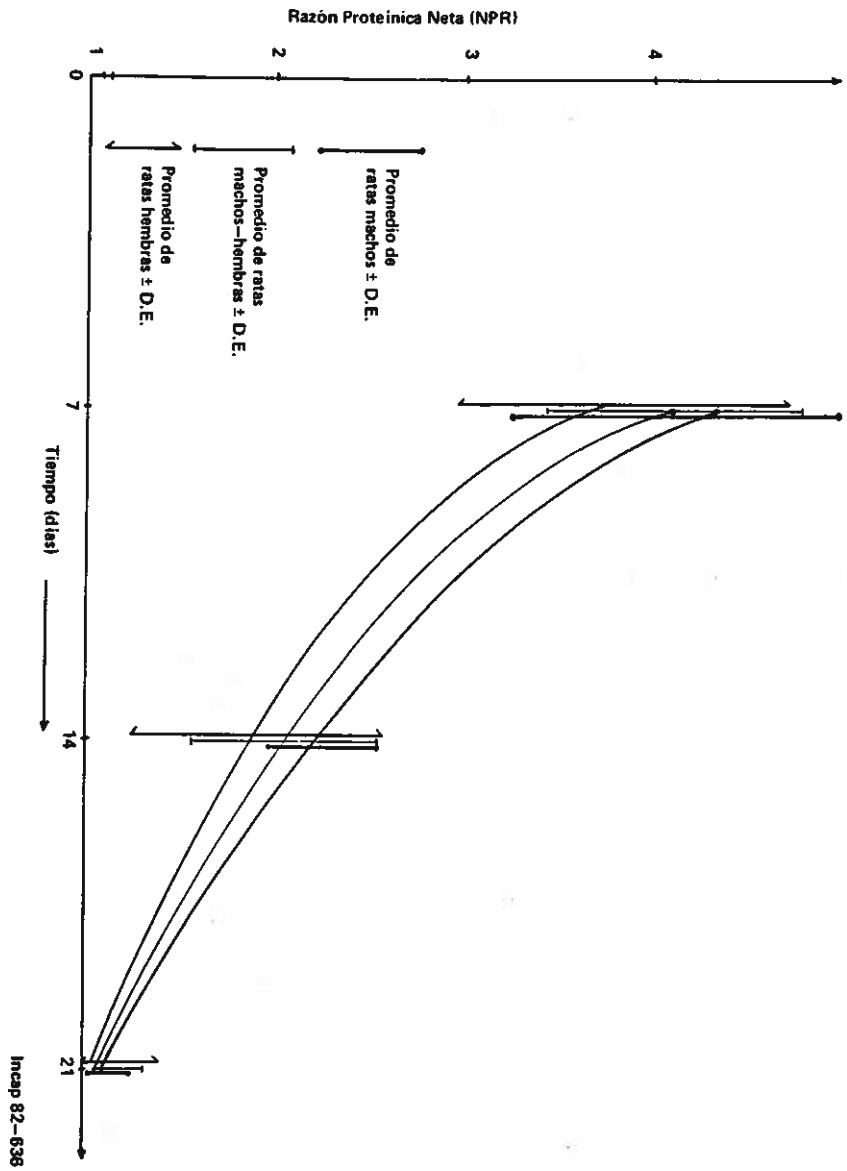
Razón Proteínica Neta (NPR)
0
1
2
3
4
Promedio de ratas hembras ± D.E.
Promedio de ratas machos–hembras ± D.E.
Promedio de ratas machos ± D.E.
Tiempo (días)
7
14
21
Incap 82–638

FIGURA 5

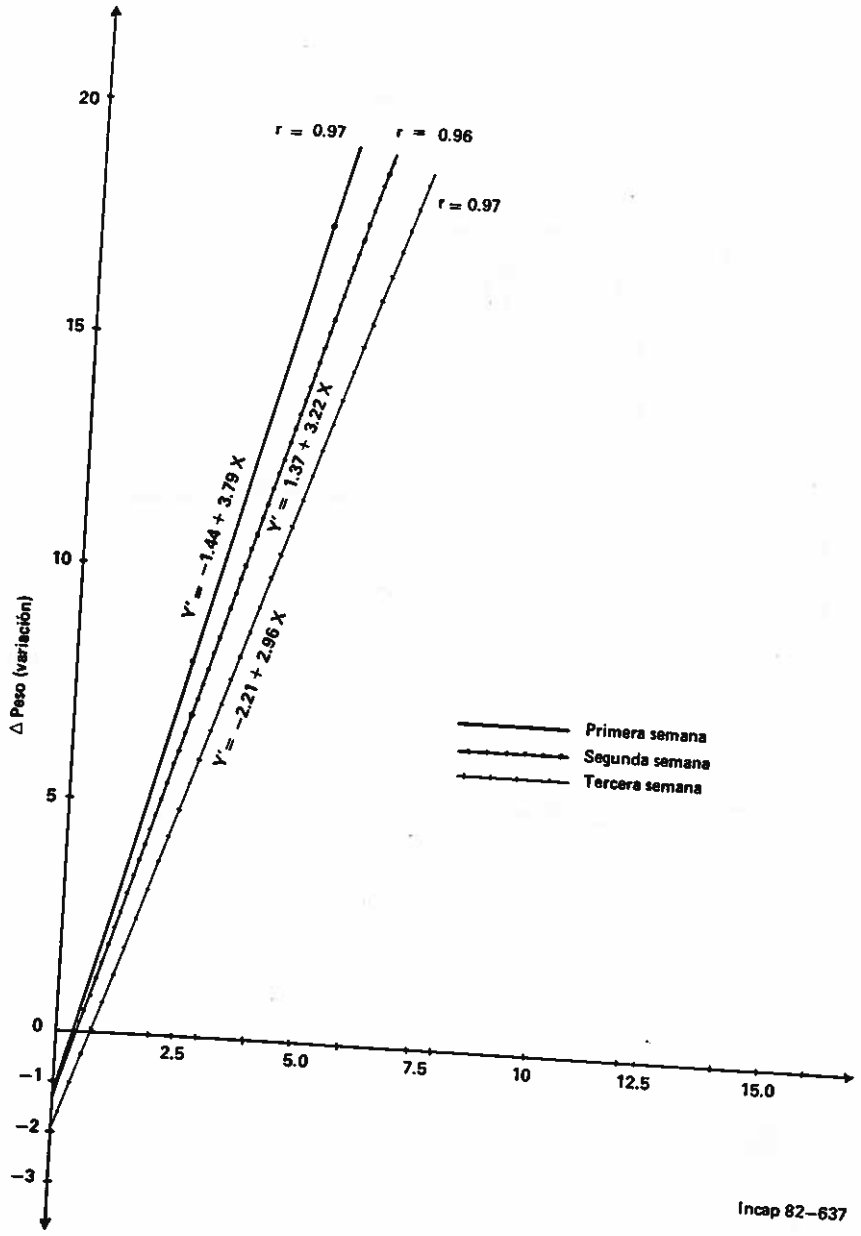
r = 0.97
r = 0.96
r = 0.97
Y' = −1.44 + 3.79 X
Y' = 1.37 + 3.22 X
Y' = −2.21 + 2.96 X
Δ Peso (variación)
20
15
10
5
0
−1
−2
−3
2.5
5.0
7.5
10
12.5
15.0
Primera semana
Segunda semana
Tercera semana
Incap 82–637

FIGURA 6

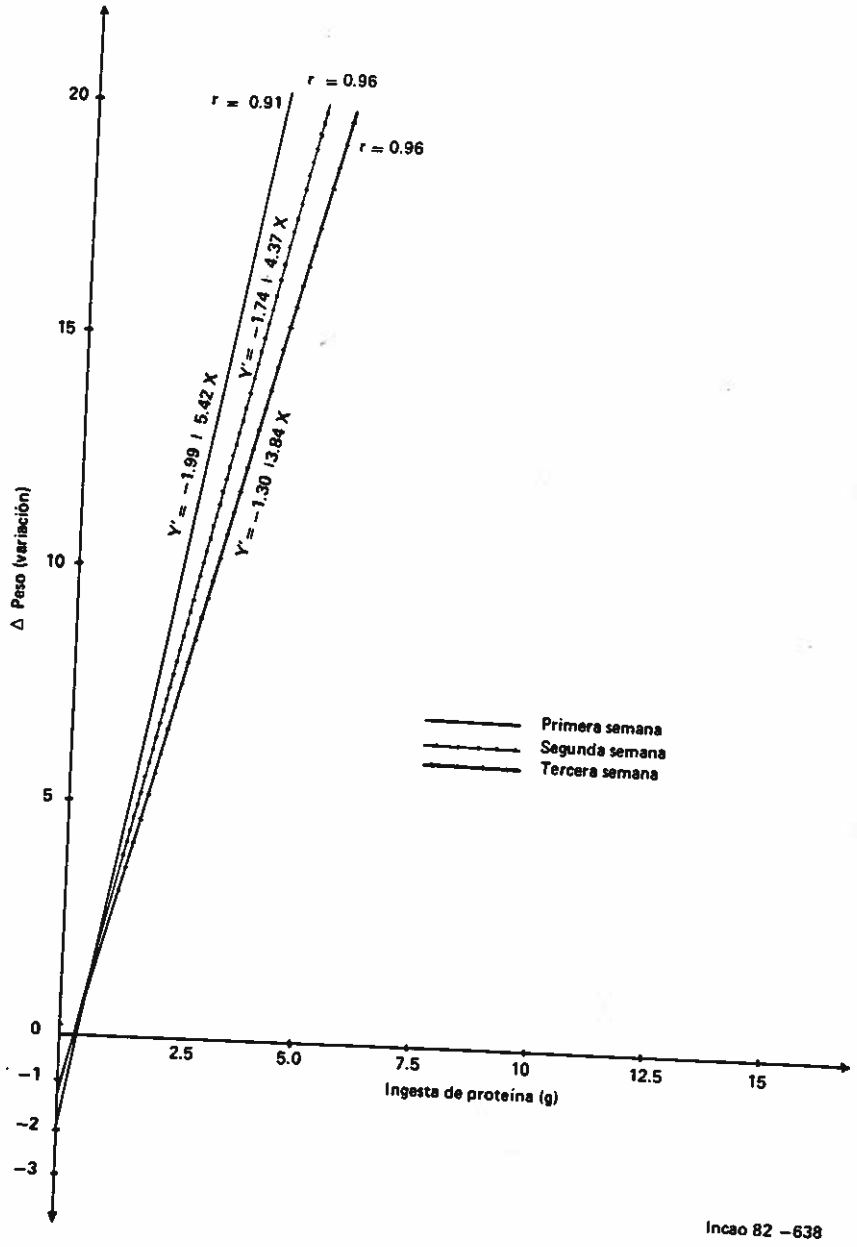
r = 0.91
r = 0.96
r = 0.96
Y' = −1.99 + 5.42 X
Y' = −1.74 + 4.37 X
Y' = −1.30 +3.84 X
Δ Peso (variación)
20
15
10
5
0
−1
−2
−3
2.5
5.0
7.5
10
12.5
15
Ingesta de proteína (g)
Primera semana
Segunda semana
Tercera semana
Incao 82 −638

FIGURA 7

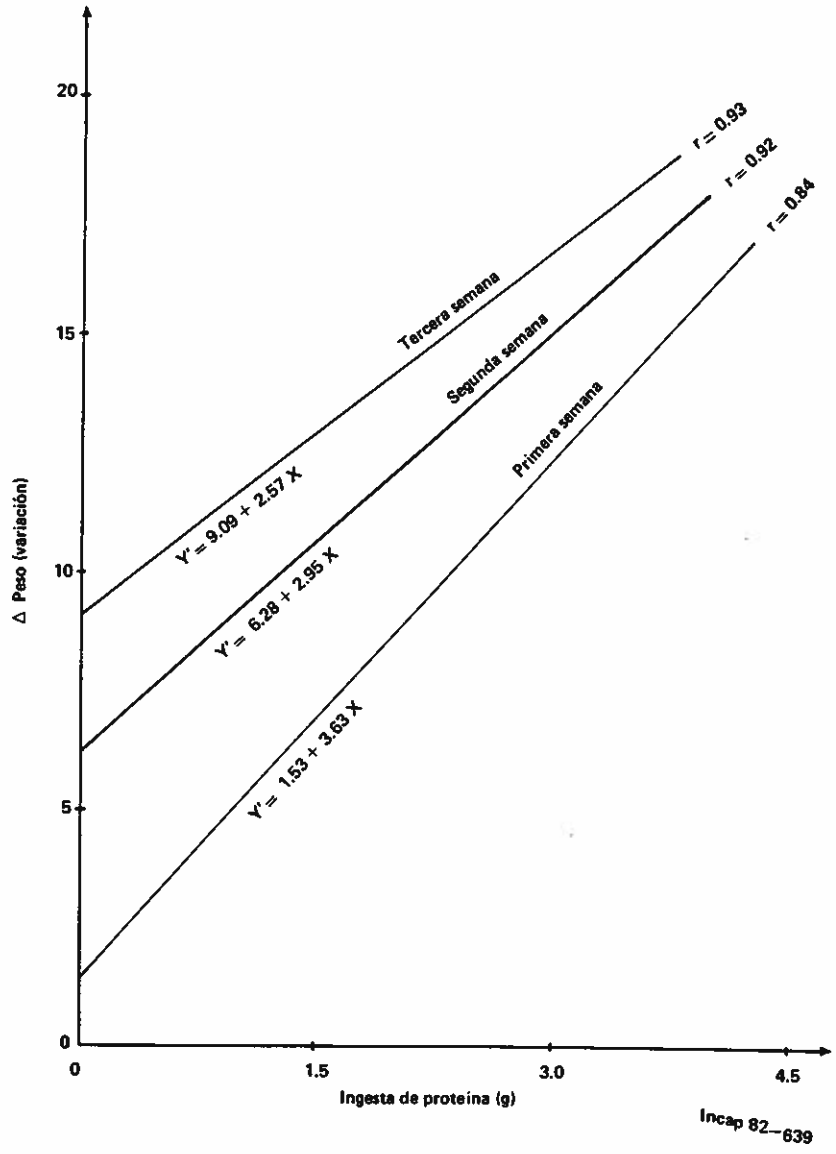
20
15
10
5
0
Δ Peso (variación)
0
1.5
3.0
4.5
Ingesta de proteína (g)
Incap 82–639
Tercera semana
Segunda semana
Primera semana
r = 0.93
r = 0.92
r = 0.84
Y' = 9.09 + 2.57 X
Y' = 6.28 + 2.95 X
Y' = 1.53 + 3.63 X

FIGURA 8

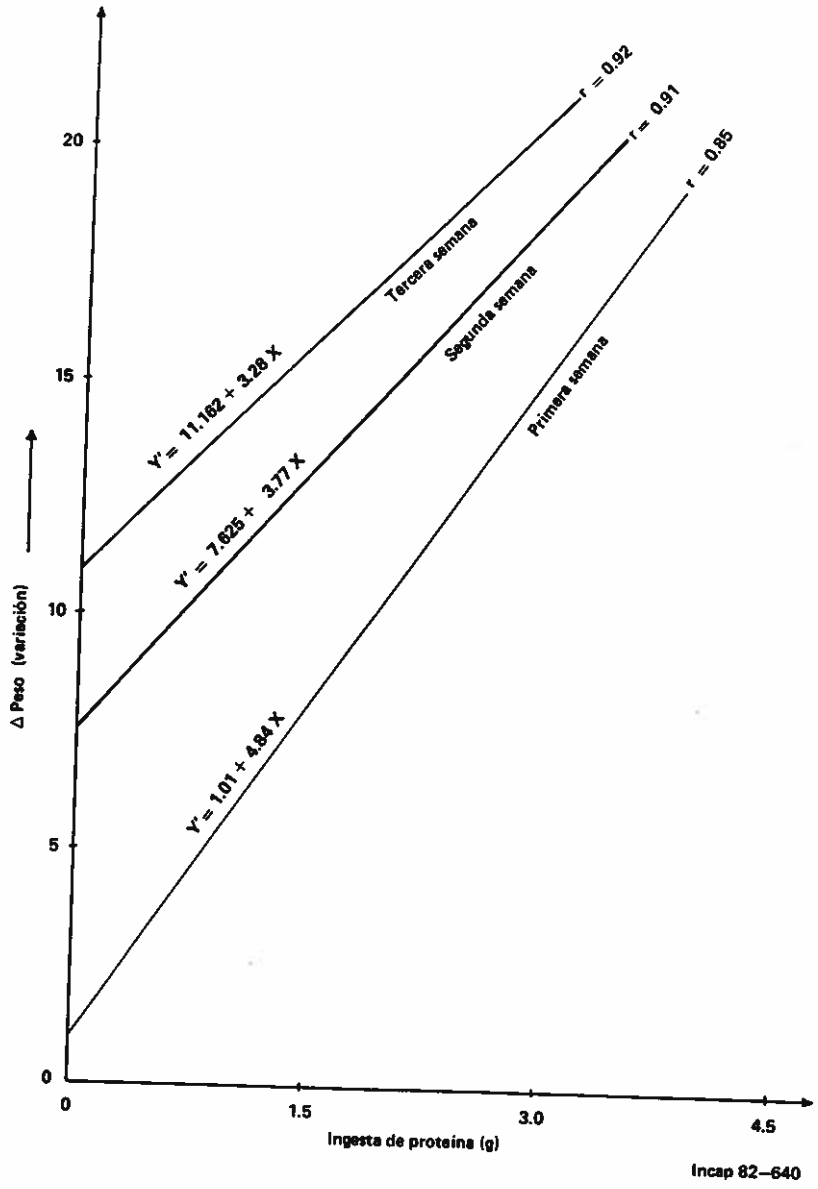
20
15
10
5
0
Δ Peso (variación)
Y' = 11.162 + 3.28 X
Y' = 7.625 + 3.77 X
Y' = 1.01 + 4.84 X
r = 0.92
r = 0.91
r = 0.85
Tercera semana
Segunda semana
Primera semana
0
1.5
3.0
4.5
Ingesta de proteína (g)
Incap 82–640

FIGURA 9

Leyenda de las Figuras:

Figura 1: Variación del PER con el nivel de proteína (Nitrógeno total considerado proteínico) derivada de harina de carne de tiburón tollo (Squalus acanthias).

Figura 2. Variación del PER con el nivel de proteína (nitrógeno por no proteíco) derivada de harina de tiburón tollo (Squalus acnanthias).

Figura 3. Variación del PER (9.97% proteína de harina de tiburón) con el tiempo.

Figura 4. Variación del NPR con el nivel de proteina (nitrógeno total, tiempo: 14 días) derivada de harina de carne de tiburón tollo (Squalus acanthias).

Figura 5. Variación del NPR (nivel de proteína = 9.97%) con el tiempo.

Figura 6. Indice de crecimiento nitrogenado (se considera el nitrógeno de la dieta como totalmente proteico).

Figura 7. Indice de crecimiento nitrogenado (nitrógeno corregido por nitrógeno no proteico).

Figura 8. Indice de crecimiento nitrogenado (nitrógeno total) según Hegsted.

Figura 9. Indice de crecimiento nitrogenado (nitrógeno corregido por NNP) según Hegsted.

PROCESSING AND QUALITY ANALYSIS OF DEHYDRATED SEAFOODS

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INTRODUCTION

Dehydration is one of the most important methods of preserving fish throughout the world. About 20 - 25 percent of the world catch for human consumption in the mid seventies was dried, salted, smoked, or treated by some combination of these processes each year (Waterman, 1976). More than half of this amount is estimated to be dried in some way either without the addition of salt or after a preliminary salting treatment.

Southeast Asia has long been one of the world's leading producing and consuming areas for dehydrated seafood products. According to Marynard (1983), total production of dried products was about 20 percent of total world production; however, the total consumption in the area exceeded production. Hong Kong has a per capita consumption of 3.8 kg and imports which total U.S. $98.4 million in value annually. This represents about 75 percent of the value of all imports in Southeast Asia. The second largest importing country is Singapore which imports products valued at U.S. $18.0 million, but most of these products are re-exported. Most of their imported dried products are supplied by other Southeast Asian countries. Hong Kong and Singapore are the high potential markets for importing U.S. dried seafoods.

In order to develop dried seafood products for export, it is necessary to match the underutilized species which can be caught in the South Atlantic and Gulf of Mexico to the dried seafoods in existing markets. The appropriate form of the dried seafoods and quality standards are also necessary information. Only a few papers (Sumardi, et al., 1982; Gopakumar and Devadasan, 1983; and Zain and Yusof, 1983) have surveyed the moisture and salt content of local products. Therefore, this study emphasized the quality analysis of commercial imported dried seafood and development of a drying process for selected underutilized species.

MATERIALS AND METHODS

Commercial Dehydrated Seafoods

Unsalted and salted dehydrated seafoods imported from seven different countries were bought from ethnic markets in Atlanta, Georgia and

Jacksonville, Florida. They were put in a large plastic bag and stored in a cooler until testing. Three samples of each product were ground in Mason jars. For large fish, only the meat was used; while small fish were used whole minus the head. The ground sample was tested for moisture content, salt content, TMA-N, TBA, and water activity (A_w).

Moisture content was measured by drying the samples to constant weight in a vacuum oven (Fisher isotemp vacuum oven, model 281) at 95°C and 30 in Hg vacuum for 6 hours.

Salt content was determined by using an Orion Chloride electrode (model 94-17 B) that was connected to a Corning pH meter (model 130). Electrode potentials were translated into a percentage of sodium chloride using a calibration curve.

A trimethylamine-specific electrode method (Chang et al., 1976) was used to measure trimethylamine-nitrogen (TMA-N) levels. An Orion ammonia electrode (model 95-10) was made TMA specific by replacing the inner filling solution with 0.01 μ TMA hydrochloride in 0.04 μ kcl. Formaldehyde was added to the sample solution to reduce the response of the electrode to ammonia. The electrode, connected to a Corning pH meter (model 130), was inserted in homogenates of fish muscle. Electrode potentials were translated into TMA concentrations using a standard curve.

An improved TBA (thiobarbituric acid) test (Blight, 1975) was used to measure the rancidity. Duplicate measurements were made and the average was expressed as μ moles Malonaldehyde/100 grams of fish (wet basis).

A hygrometer (model 15-3050, American Instrument Co., Silver Springs, MD) with sensors was used to measure the water activity (A_w). Ten grams of samples were ground in a Mason jar and the relative humidity measured by inserting the proper sensor. The sample equilibrated for one hour at constant temperature (25°C). Conversion of the hygrometer dial reading to relative humidity was done using a calibration curve, the value divided by 100 is the water activity (A_w). Sensors were calibrated with saturated reference salt slushes before measurement.

Dried samples were immersed in water at room temperature for 7 hours. The increased weight divided by the final weight is expressed as percent rehydrability.

Raw Material for Drying Test

Whiting, Menticirrhus americanus, (about 0.5 lb. each); sea trout, Cynoscion nebulosus, (about 1.0 lb. each) thread herring, Opisthonema oglinum, (about 15-20 gm. each); and striped mullet, Mugil cephalus, (about 1.5 lb. each) were selected for this study. All fish, except thread herring were bought from local fish markets in Brunswick, Georgia. The thread herring was obtained from a bait dealer in Florida.

Whiting and sea trout were split, gutted, and scaled. Thread herring were either split, gutted and scaled or gutted and scaled without splitting. Mullet were split, gutted and deboned with scales on and without scales. In addition, mullet were salted by soaking in a saturated brine solution for five days until final salt content reached approximately 20%.

A closed system dryer with controlled temperature, relative humidity and air velocity was used to dry the fish. Optimal drying conditions were determinated for different species and treatments. To facilitate changing the temperature, relative humidity and air velocity for different drying stages according to the evolving status of tissue being dried, a small programmable controller (CZ-1000 W, Omron Electronics) was used. It has 16 input and 16 output ports and has a memory capacity of 256 word RAM and a 4 x 256 word EPROM in relay ladder language. Staged drying, therefore, was automatically conducted within a single drying chamber. A load cell was used to record the weight loss of the fish during the drying process. It provided a guide to the drying status.

RESULTS AND DISCUSSION

Quality Analysis of Commercial Dehydrated Seafoods

The general description of imported dried salted seafoods which are available in serval ethnic stores is shown in Table 1. Dried fish were as long as 40 cm and as short as 8 cm. The names of the products were copied form the packages. Large fish such as yellow fish, salted fish and mackerel were sold individually by weight, while medium and small fish were packaged in either 8 oz or 16 oz units. Products were not packaged well, except anchovies which were packaged with nitrogen. Yellow fish were not even packaged. Product forms included whole fish, whole gutted fish, headless gutted fish, split fish and fillet. Most of the dried salted fish had a strong fishy odor, and some had a brownish oxidation color. Unsalted dried fish had a better appearance and no fishy odor (Table 2).

Moisture content, salt content, and water activity of dried salted seafoods are important measures of product quality and stability, but commercial dried seafoods from Southeast Asian countries had a wide range of moisture and salt content. Moisture contents of large fish (>35 cm), medium fish (20-35 cm), small fish (<20 cm) and squid were 40-54%, 38-47%, 15-40% and 17-21% respectively. Salt contents were 15-22%, 15-17%, 4-17% and 6-11% respectively (Table 3). There was a lowering of moisture content and salt content with decreasing fish size. This trend was similar to the report of Zain and Yusof (1983), but the large and medium dried fish of Malaysia had a lower moisture and a higher salt content. Gopakumar and Devadasan (1983) reported that the moisture content and salt content of most Indian dried salted fish were 30-45% and 15-35% respectively. While Sumardi and his associates (1982) stated that the moisture content and salt content of Indonesian products were

45-51% and 22-38% respectively.

The Product quality and stability of dried salted fish is closely related to the water activity (Waterman, 1976; Rockland and Nishi, 1980). Most spoilage bacteria will cease to grow in a food with water activity (A_w) below 0.9. The growth of molds is inhibited below 0.8 and halophilic bacteria do not grow below 0.75. Zain and Yusof (1983) reported that selected Malysian products had an A_w value of 0.75 for all large and medium fish and 0.56-0.80 for small fish and squid. In this study, the A_w of dried salted fish ranged from 0.74 to 0.87 (Table 3), while the A_w of large squid and small squid are 0.64 and 0.72 respectively. The A_w of unsalted fish ranged from 0.69 to 0.76 and the moisture contents varied from 12 to 19% (Table 4). Unsalted commercial products seemed to be more stable than the salted products.

The TMA-N and TBA values are also shown in Tables 3 and 4. TMA-N values for salted dried fish was higher than that of unsalted products except sepat and anchovy. Since the formation of TMA is related to many factors such as different species, bacterial growth, processing methods, and storage conditions (Hebard et al., 1982), it was difficult to find the main reason for the high TMA-N values in these commercial products. However, it was not hard to realize that sun-drying processes, poor packaging, and improper storage will decrease the product quality.

The TBA values of oily fish such as mackerel, sardine and anchovy were higher than that of others. These products had an oxidized appearance and a strong rancid odor.

Heavily salted dried fish had rehydrability which ranged from 17 to 19%, while unsalted products varied from 51 to 54% (Table 5). It seemed that the higher the salt content the lower the rehydrability.

Optimal Drying Conditions of Selected Fish

Underutilized species such as whiting, sea trout, thread herring, and mullet were selected to produce dried products which are similiar to commercial products. Except for the air velocity which was fixed at 250 ft/min, the air temperature and relative humidity were changed depending on the species and surface condition of the fish during the drying process.

Two drying conditions, one being a single-stage and the other a two-stage drying process, were acceptable. The two-stage method utilized a 40°C air temperature and 20% Rh for 2 hours then 35°C and 45% Rh for the remaining drying time. Split head-on whiting took 48 hours to loss 70% of its original weight with this method. The final moisture contents for the side without bone and the side with bone were 13.5% and 19.5% respectively. When the single-stage method which is 35°C and 45% Rh continuously was used, it took 90 hours to reach 70% weight loss (Table 1). There was a 10% difference in rehydrability between the side of the

fish with bone and without bone of the two-stage dried split fish (Figure 2). When comparing the different drying conditions, the two-stage dried fish showed the highest rehydrability (Table 6).

Sea trout lost 70% weight in 96 hours using a two-stage drying process which is the same drying condition for drying whiting. Final moisture contents of dried products for the side without bone and the side with bone were 14% and 18.5% respectively. The rehydrability of both sides was 38.37%.

A single-stage method with lower temperature at 24°C and 45% Rh was used for drying thread herring. Split herring took 30 hours to lose 67% and 40 hours to lose 70% weight (Figure 4). Whole gutted herring took 90 hours to lose 67% of the weight. The moisture contents of dried split fish and whole gutted fish averaged 12.5% and 14.3% respectively.

The lower air temperature of 24°C was also used for drying mullet, the oily fish. The two-stage method utilized a 35°C air temperature and 20% Rh for 2 hours then 24°C and 45% Rh for the remaining drying time. The scaled unsalted mullet took 118 hours to get a 67% weight loss which equaled a final moisture content of 14% (Figure 6). A 60% weight loss was observed for the mullet with scales and a moisture content of 18% in the same drying time. Salted mullet with scales on and scales off had a 4% difference in the final weight loss. The moisture contents for mullet with scales and scaled mullet were 27% and 23% respectively. The fish with scales had a better apperance while scaled mullet has oil seepage from the skin. These tests suggest that oily fish such as mullet are better processed as salted dried product.

ACKNOWLEDGEMENTS

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Table 1. The general observation of dehydrated commercial salted seafoods.

Product	size (cm)	Form	Origin of Product	Observation
Salted fish	40	Deheaded, gutted	Venezula	Firm, strong fishy odor
Yellow fish	40	Deheaded, gutted	Hong Kong	Firm, without package
Mackerel (L)[a]	36	Whole, gutted	Thailand	Soft, strong putrid odor
Croaker	20	Whole, gutted	Hong Kong	Firm, strong fishy odor
Nemipterids	23	Split, gutted	Philippines	Firm, smoked flavor
Mackerel (M)	20	Split, gutted	Philippines	Soft, rancid/fishy odor
Sepat	15	Deheaded, gutted	Thailand	Firm, dark brown color
Herring	13	Whole	Philippines	Soft, fishy odor
Sardine	15	Whole	Philippines	Soft, brown color
Anchovy	8	Whole	Japan	Firm, packed in N_2
Bacalao	16	Fillet, section	Canada	Soft, wet
Squid (L)	28	Split	Thailand	Firm, strong fishy odor
Squid (S)	10	Split	Thailand	Soft, strong fishy odor

[a] (L):Large; (M):Medium; (S):Small

Table 2. The general observation of commercial dehydrated unsalted seafoods.

Product	Size (cm)	Form	Origin of Products	Observation
Pollack	22	Whole, gutted	Korea	Firm, vacuum packaged
Stock fish	25	Fillet, skinned	Hong Kong	Frim
Eel	10	split, cut section	Hong Kong	Firm
Filefish	24	Fillet, skinned	Korea	Firm

Table 3. The moisture content, salt content, TMA-N, TBA, and A_w of commercial dehydrated salted seafoods.

Product	Moisture Content (%)	Salt Content (%)	TMA-N (mg/100g)	TBA (μm MA/100g)	A_w
Salted fish	51.45±2.11	16.02±1.11	184.17±23.55	1.16±0.99	0.87
Yellow fish	43.20±3.13	19.27±1.63	25.33±6.11	3.22±0.72	0.85
Mackerel (L)	41.89±1.84	17.50±0.50	121.10±12.73	3.90±0.84	0.76
Croaker	44.05±0.53	15.85±0.95	110.74±62.42	2.27±0.89	0.84
Nemipteriods	40.94±2.23	16.35±1.08	57.56±1.64	6.48±2.25	0.82
Mackerel (M)	46.65±0.38	16.91±0.23	57.04±4.41	26.30±1.28	0.81
Sepat	36.49±2.83	15.86±0.28	8.17±0.92	5.05±0.98	0.82
Herring	38.91±0.45	12.76±0.24	53.45±4.32	5.90±1.10	0.79
Sardine	38.85±0.58	12.79±0.73	31.47±3.22	10.83±0.32	0.81
Anchovy	15.39±0.14	4.24±0.10	15.63±3.61	15.91±0.88	0.74
Bacalao	53.01±1.31	22.41±0.99	22.70±4.23	0.27±0.49	0.87
Squid (L)	17.32±0.13	6.83±0.78	50.23±4.69	7.82±1.77	0.64
Squid (S)	21.07±0.24	9.86±0.71	76.69±2.95	11.26±1.67	0.72

* All data were the average of three samples

Table 4. The moisture content, TMA-N, TBA and A_w of commercial dehydrated unsalted seafoods

Product	Moisture Content (%)	TMA-N (㎎/100g)	TBA (μmMA/100g)	A_w
Pollack	13.09±1.26	19.48±6.14	7.11±0.89	0.70
Stock fish	14.04±0.42	13.95±6.65	2.41±0.22	0.72
Eel	13.11±0.50	21.03±4.53	1.97±0.60	0.69
Filefish	18.84±0.78	21.97±3.73	3.69±1.71	0.76

* All data were the average of three samples

Table 5. The rehydrability of commercial dehydrated seafoods

Product	Classification	Rehydrability (%)
Yellow fish	salted	17.91
Croaker	salted	17.35
Nemipterids	salted	19.06
Sardine	salted	19.21
Anchovy	lightly salted	43.38
Squid (L)	lightly salted	47.43
Squid (S)	lightly salted	47.92
Pollack	unsalted	53.85
Stock fish	unsalted	62.41
Filefish	unsalted	51.31

* All data were the average of three samples

Table 6. The rehydrability of whiting at different drying conditions

Drying Conditions	Portion without backbone	Portion with backbone	Both Sides
40°C, 20% Rh for two hours then change to 35°C, 45% Rh	49.03%	40.12%	43.74%
35°C, 45% Rh	46.64%	38.76%	42.20%
24°C, 45% Rh	49.13%	36.50%	41.20%

* All data were the average of three samples

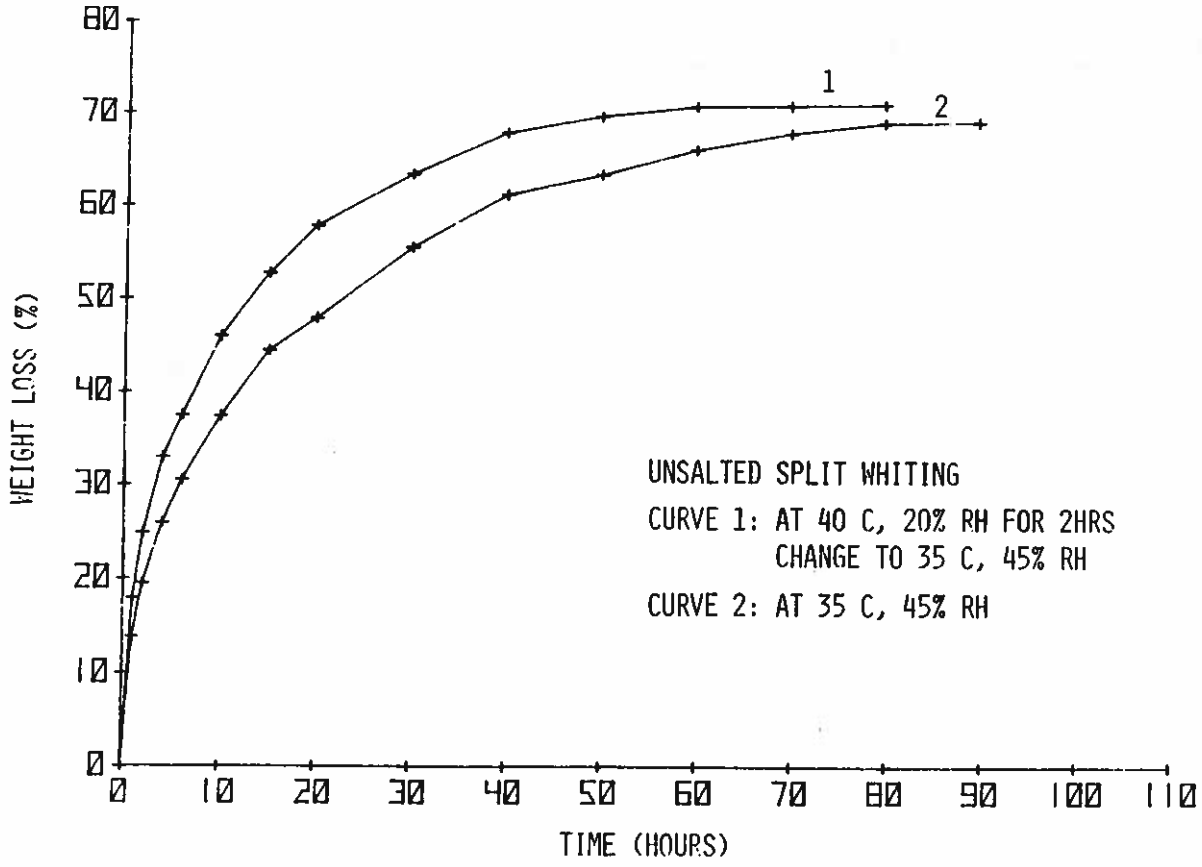

Figure 1. The drying curves of whiting at different drying conditions.

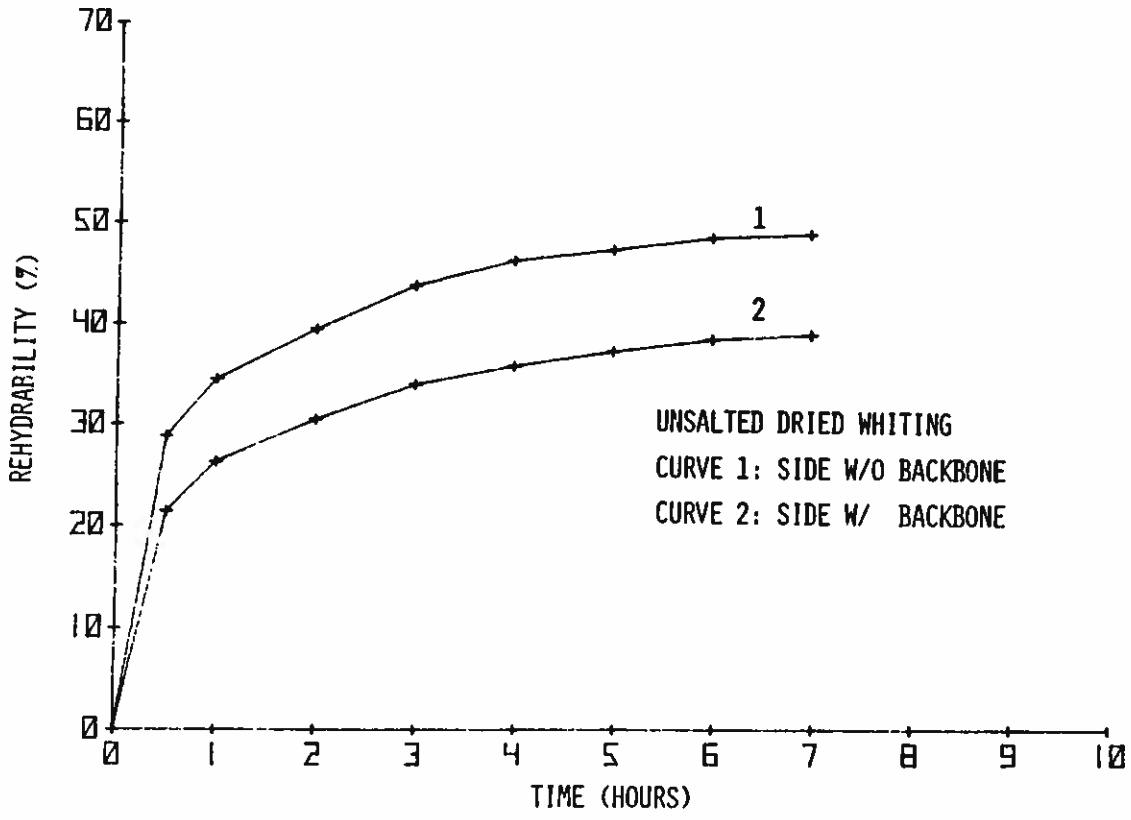

Figure 2. The rehydrability of dried whiting.

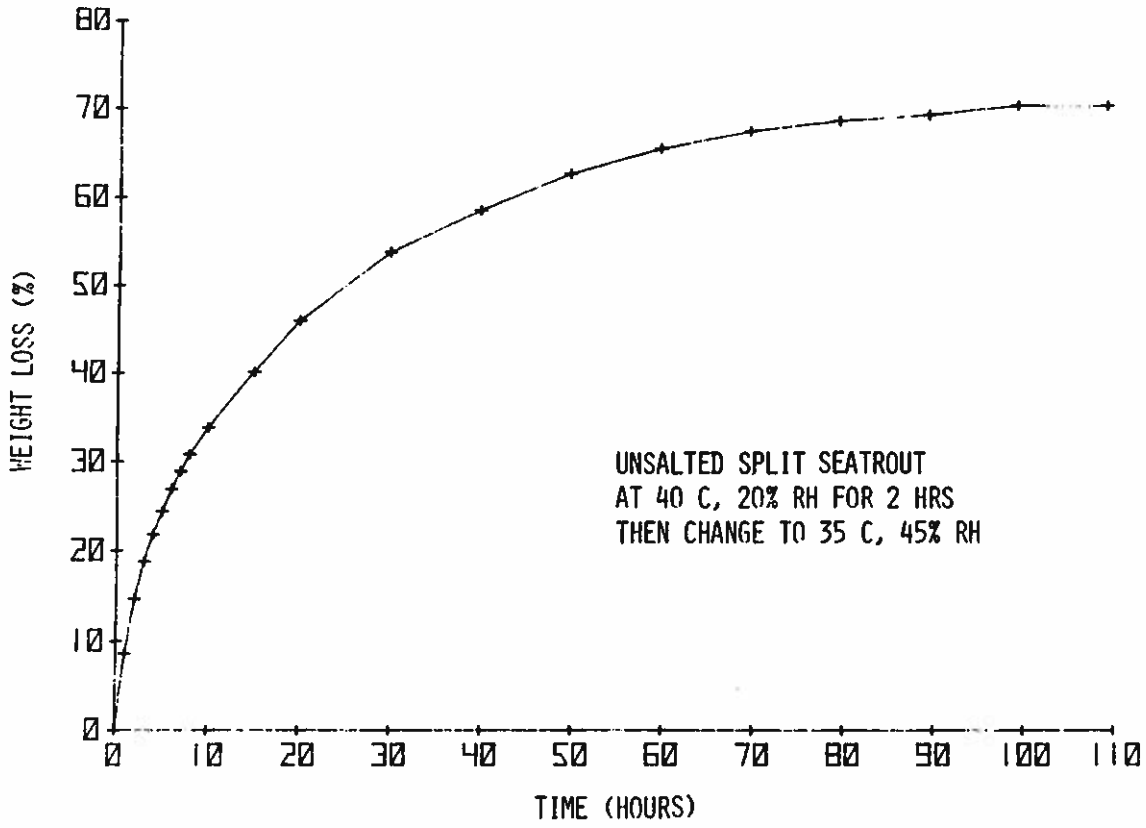

Figure 3. Thd drying curve of sea trout.

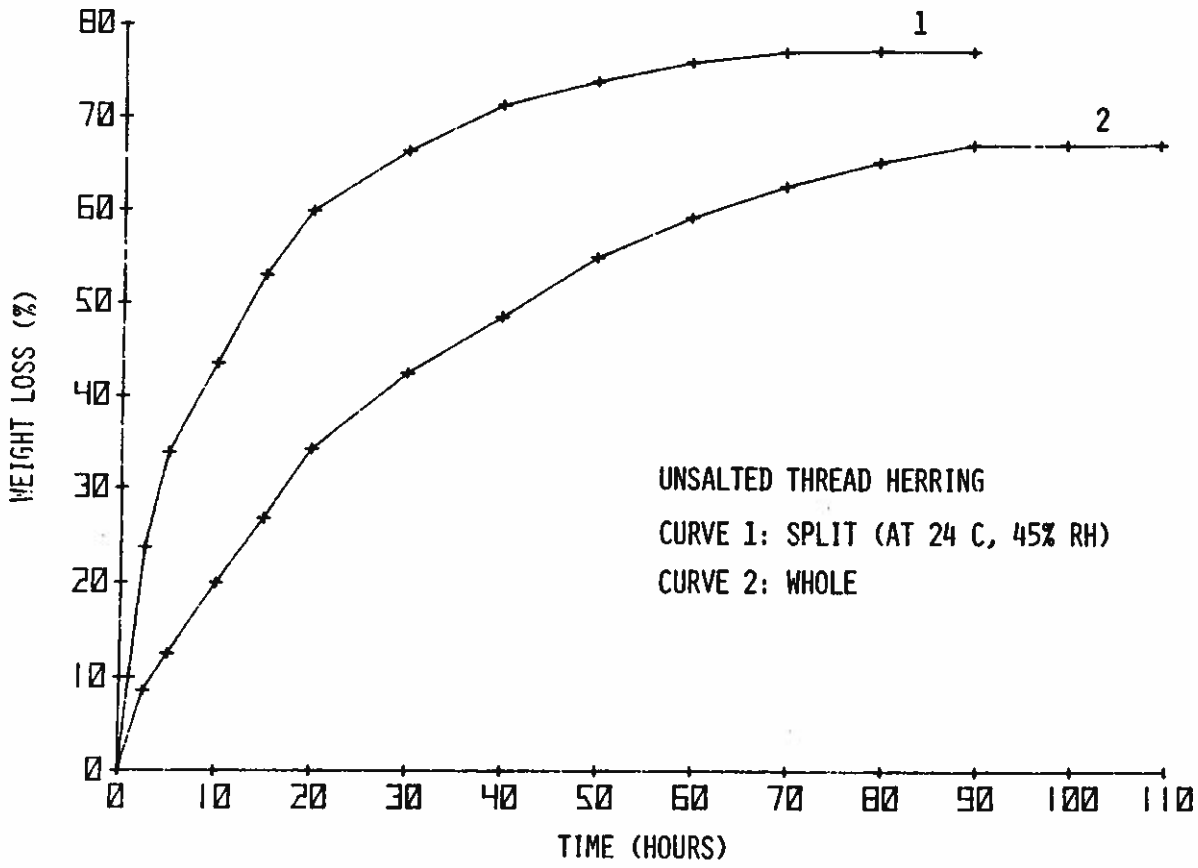

Figure 4. The drying curves of thread herring with different treatments.

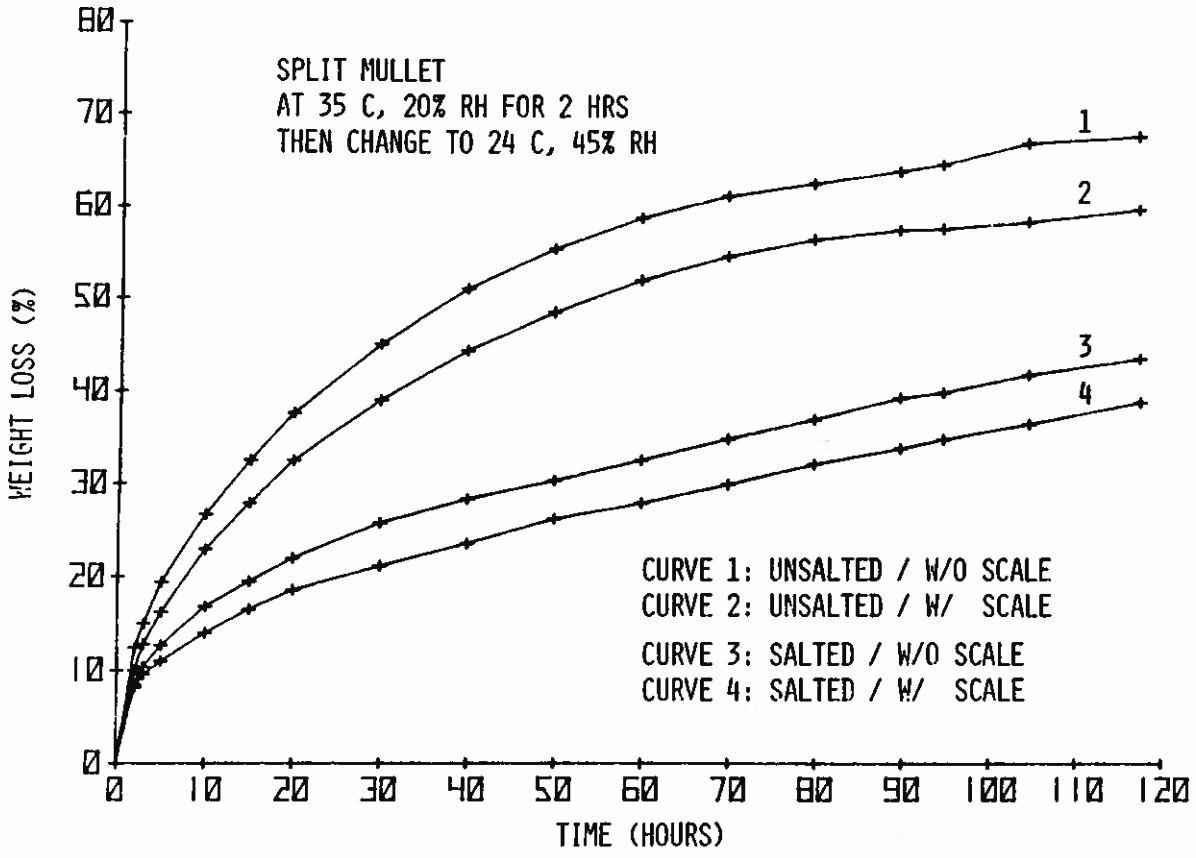

Figure 5. The drying curves of mullet with different treatments.

MARINE EXTENSION SERVICE
UNIVERSITY OF GEORGIA

SOUTH YARDS P. O. BOX ~~XXX~~ Z BRUNSWICK, GEORGIA ~~31522~~ 31523 (912) 264-7268

February 7, 1984

Ranzell Nickelson II
Marine Project Supervisor
442 Kleberg Center
Texas A & M University
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Dear Dr. Nickelson,

Enclosed please find a copy of the corrected references which are part of my paper entitled "Processing and Quality Analysis of Dehydrated Seafoods". Would you please substitute the enclosed for the one which I have already submitted at the Annual Meeting of the Tropical and Subtropical Fisheries Technological Conference last month in Brownsville, Texas.

Thank you very much for your assistance.

Sincerely,

Yaowen Huang

Yaowen Huang

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PRODUCT PLANNING AS A MARKETING TOOL: USING SHARK IN JERKY PRODUCTS

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INTRODUCTION

Product planning is a long term investment decision generally made by top management. This decision requires the commitment and use of resources, people and time. Planning activities required to establish a new product in the marketplace are developing the product, testing its performance, systematically analyzing how the product is perceived by ultimate consumers and the trade, determining which promotion strategy most efficiently stimulates purchases and what the competitor's response will be to the product's introduction. Planning for the introduction of new products has been described as the transition of having an insight, turning it into an idea and developing a series of action plans with the objective of ending up with a satisfactory performer in the market (Smallwood, 1977).

THE PROCESS OF PLANNING FOR NEW PRODUCTS

Product planning is perhaps the most important long term decision a firm can make because:

1) a new product offers an excellent investment opportunity by providing
   a) the firm greater strength in the market by selling a more complete line;
   b) a competitive advantage;
   c) a product which meets a current need (or creates a need).
2) the cost of new product planning and development is high with no immediate payoff.
3) there is a high risk of the new product's failing to meet expectations. This is particularly true in food products because of the long term testing required to accurately assess consumers' taste preferences.

Product development and planning strategies can be characterized as true innovation, adaption or imitation (Morris, 1964). True innovation, if successful, may set standards for the industry. Developing and succeeding with a true innovation will provide the firm

with greater net profit than will adaptation or imitation. However, the risks of developing and succeeding with a new product are disproportionately high. For this reason true innovation will probably be left to the giants in the industry because they have the capability of absorbing losses from failures.

The imitator may be contemplating a product which is new to their line, but which currently exists in the market. An example would be for a firm to begin producing and marketing a line of battered fish portions cut from frozen blocks. Payoffs from imitations are not nearly as high as with true innovation but neither are the risks since an established market already exists.

Adaption is essentially a process of modification. Adaption may substantially improve upon a product by substituting different raw materials into a known process to produce a slightly different product which compliments an existing line. Of the three categories of product planning: innovation, imitation or adaption, the latter offers the best opportunity for achieving planned sales volumes and financial returns (Morris, 1964).

In the food processing industry adaption is important. Aside from the "new and improved" claim, the substitution or alleviation of ingredients in existing products is widespread. Examples include the development of low salt canned vegetables, decaffinated coffees and low sugar canned fruits. The rationale for this practice may partially be explained by food manufacturers' seeking to meet the needs of distinct, segmentable groups which comprise the total market for food products.

Within the seafood utilization industry the process of planning and developing new products has lagged behind the red meat and poultry industries. Several reasons may be the size of most firms in the industry, the variability in supply of suitable seafood products as raw materials and to some extent limited, traditional markets for output.

IS THERE A FUTURE FOR SHARK AND SHARK PRODUCTS

With the exception of Mako (*Isurus Otyrinchus*), there is relatively low demand for shark and shark meat products. Furthermore, shark has not been held in very high esteem by many Texas seafood processors. Three years ago one firm attempted to market shark bycatch but discontinued after finding resistance in the trade. Despite these negative perceptions and experiences, many processors and boat owners admit that the potential for shark utilization is good, but current conditions are unfavorable.

Part of the reason for perceived, unfavorable conditions is that shark has had a reputation as a product which has been difficult to handle. Over the past three years Texas A&M University has been involved in a shark utilization project. Based on detailed but common sense on-board handling requirements, this project has demonstrated that high quality shark can be delivered to the dock from offshore fishing trips. Studies on frozen storage stability have

shown that with proper freezing and glazing procedures low drip loss can be achieved and high quality defrosted meat is available; suitable as a raw product prepared by conventional means such as soups, kabobs, grilled steaks, etc., and also as raw material for hot smoked portions and jerky (Finne and Miget, 1983).

Park of this project has centered around developing new products from shark. In preliminary taste tests, participants were enthusiastic about eating a new snack jerky. Therefore, we feel like shark-based jerky has good potential as an addition to the shelf stable, ready-to-eat snack meats line currently available in convenience outlets.

High quality jerky can be manufactured from sharks held in long term frozen storage using accepted industry practices. The process is outlined below.

THE JERKY PROCESS

Frozen, glazed shark tubes (*Carharhinus falciformis* and *C. obscurus)* were partially defrosted and hand butchered (steaked, trimmed and sliced) into strips approximately 1/4 inch thick and one inch wide. Length ranged from one to 6 inches.

These strips were placed in a solution of teriyaki sauce, canning salt and spices. Salt was added in the amount of 5% by weight of the teriyaki sauce, shark strips were added and the combination was left to cure overnight for about 15 hours at approximately 36°F. The cured strips were removed from the lug, placed on a rack and rinsed. The rinsed strips were then placed in the smoker and dried for 1/2 hour at 120°F. After the initial drying phase, the temperature was increased to 160°F., wet wood chips were added to the charcoal fire in the smokebox and the product cooked for approximately 2 hours 20 minutes. The wet bulb thermometer reached a temperature of 155°F. Jerky was removed from the smoker and placed under refrigeration until vacuum packaged.

Subsequently, salt and moisture analyses were performed which indicated an average remaining moisture content of 38.69% and a salt content of 4.733%. Using the formula (% salt/% moisture + salt) x 100, jerky cured with 5% salt had a waterphase salt value of 10.899% which is within FDA guidelines for fish jerky products.

CAN A JERKY PRODUCT SUCCEED?

Angelus (1969) has suggested that success with new products is dependent upon the combination of product-oriented characteristics and market conditions and situations. While this appears common sensical, the point made is that historically new product opportunities have existed when a manufacturer has developed a product which is in fact different and when the marketing channel finds impetus to

aggressively sell the product. As an example, assume that a manufacturer develops a new product but the price is too high for any reseller to squeeze more than a few pennies of gross margin out of it. The result of this condition will be a "good" product which never gets the exposure it needs simply because there is no incentive for resellers to handle it as part of their product mix.

Particularly with food products which have a long marketing channel, the lack of gross profit for resellers may play more of a role in affecting the products's exposure in the market than whether the product in the market is superior.

Whether a shark jerky product will succeed is dependent upon a number of variables. However, evaluating shark jerky in light of current market situations and conditions may provide some indication of its performance

1. Shark jerky will retail for approximately $.89-$.99 per ounce. This price is actually below the market price for natural, sliced beef jerky. Additionally, a patron's sampling a relatively low-cost shark item may reduce purchase risk when ordering other shark items.
2. Judging from the width and length of the ready-to-eat, shelf-stable snack meat product line and its prominent placement in convenience stores, the demand for these products appears to be quite high.
3. With the price of substitute products quite high, good gross margins are obtainable. Reported gross margins for natural beef jerky are 33%. Estimated gross margins for natural sliced shark jerky are 50%. This should induce placement in the outlet which contributes to impulse sales.
4. In a convenience outlet product placement and limited point of purchase materials serve as promotion. Therefore, the ratio of advertising and promotional expense to sales is low. This prevailing market condition can make a particular market ripe for a new product (Angelus, 1969).
5. The two fastest moving products in convenience stores are gasoline and ready-to-eat foods.
6. Based on hypothetical cost estimates and meat and cooking yields it appears as though everyone in the marketing channel will be able to make money. This should provide some incentive to push the product through the channel.
7. Despite the demand for snack jerkys, there have been few (if any) innovations in fish-based snack products in Texas.

In summary, if current market conditions and situations indicate a product's potential, then shark jerky has a chance because 1) there is enough margin available to all members of the channel which will provide the incentive to aggressively sell the product and 2) other shelf-stable snack meats are currently enjoying good movement in convenience stores.

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REGION, MARKET OUTLET AND PRODUCT FORM DISTRIBUTION OF GULF OF MEXICO LANDED GROUPER

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Gulf of Mexico grouper landings account for approximately 85 percent of U.S. grouper landings. Throughout the decade of the 1970s Gulf landings were relatively stable with a range from 5 to 7 million pounds annually. Since 1980 landings have increased to over 12.0 million pounds in 1982. Florida landings (including Florida South Atlantic landings) accounted for 87 percent of total U.S. grouper landings during the 1980-82 period.

Little formal research has been conducted on market distribution patterns for the grouper industry. Such research is necessary to identify consumers of grouper seafood products and to analyze market efficiency in the marketing and processing segment of the industry. Additionally, such information is often needed by public management agencies for a variety of reasons, such as for the development of the Gulf of Mexico Reef Fish Management Plan. In fact, the process of developing that plan led to the research reported in this paper. The data for this study are based on annual surveys of principal grouper dealers located on the Gulf of Mexico coast during the 1979-82 period. Dealers who initially buy from fishermen and resell in the market place were interviewed and are referred to as "coastal dealers" in this paper. A list of twelve major coastal dealers of groupers was obtained from statistical agents of the National Marine Fisheries Service. Each dealer was initially contacted to provide information for the analysis. The response rate over the four year period varied from eight to eleven firms. Those firms responding had market shares totalling between 16 to 40 percent of total Gulf production on an annual basis. Throughout the four year period the response rate averaged 29 percent of Gulf of Mexico grouper production.

Market distribution is considered with respect to three market dimensions; region, type of buyer and product form. Sales were grouped into two regions based on states reported by coastal dealers as regions where sales were made. Sales were reported to only eleven states. The South was defined to include Texas, Florida, Georgia, Alabama and Mississippi. The Northeast included Illinois, New York, Maryland, Ohio, Michigan and Pennsylvania. Product forms reported include both fresh and frozen fillets and both fresh and frozen whole (gutted) grouper products. Types of buyers included wholesalers, retail market buyers and restaurants.

MARKET DISTRIBUTION BY REGION AND TYPE OF BUYER

Regional distribution of grouper sales suggests grouper is a seafood product largely marketed for consumption in the South. During the 1979-82 four year period, coastal dealers shipped on the average 84.4 percent of sales in terms of value and 83.6 percent of sales in terms of round weight volume to buyers located in the South (Table 1). A relatively small amount of grouper, approximately 2.2 percent, was exported by coastal dealers. The remaining 13 to 14 percent of sales were made to buyers located in the Northeast.

The majority of grouper sales by coastal dealers were to U.S. wholesalers, as has been found for many other seafood products (Moore, 1978 and Gillespie and Gregory, 1971). In total, 62.9 percent of dollar sales and nearly 66 percent of round weight sales were made to U.S. wholesalers (Table 1). Nearly 37 percent of dollar sales and 32 percent of sales on a round weight basis were made to retailers. The small amount remaining represents export trade. This proportion of grouper sales by coastal dealers to retail markets is relatively large when compared to sales of other species. For example, Gillespie and Gregory (1971) found that only 8 percent of Texas fresh finfish were sold by coastal dealers directly to independent retailers. A more distinguishing factor is that over 34.5 percent of all coastal dealer sales of grouper were made directly to restaurants which accounted for approximately 94 percent of all retail sales. This is consistent with Moore's (1978) findings that a few Florida east coast dealers reported as much as 70 to 80 percent of their grouper, red snapper and pompano sales made directly to restaurants.

The type of market outlet buying from coastal dealers differs considerably in terms of sales to the Northeast or South. Nearly all sales to the Northeast were to wholesale markets with 81 percent being made to wholesalers located in the New York fish market. On the other hand, 42.7 percent of sales volume to the South were made to retail outlets, with nearly all of these sales made directly to restaurants. It appears that secondary wholesalers within local market areas in the South are relatively less important to coastal dealers at the product's point of origin. This is consistent with findings by Gaston and Storey (1967) in their study of fish sales originating from Boston Fish Pier landings.

MARKET DISTRIBUTION BY PRODUCT FORM

Fresh whole (gutted) iced is the predominant product form in which grouper was shipped by coastal dealers with 63.5 percent of dollar sales shipped in this product form (Table 2). Nearly 35 percent of dollar sales were fresh fillets. Less than 2 percent of total dollar sales of grouper was frozen.

Product form demanded varied considerably depending upon type of market outlet. The survey results indicated that wholesalers predominantly demanded a fresh whole iced product compared to a

Table 1.--Percentage distribution of dollar and round weight grouper sales by coastal dealers[a] made to specified regions and types of buyers, 1979-82 average.

Region and type of buyer	Total		South		Northeast	
	Dollars	Round weight[b]	Dollars	Round weight	Dollars	Round weight
	percent[c]					
Region:						
Northeast	13.4	14.1				
South	84.4	83.6				
Export	2.2	2.3				
Type of buyer:						
Total wholesale:	62.9	65.8	57.3	62.0	99.4	99.3
New York	2.5	11.4			81.0	81.0
Other	60.4	54.4	57.3	62.0	18.4	18.3
Total retail:	31.9	31.8	42.7	38.0	.5	.6
Markets	2.4	2.4	2.7	2.8		
Restaurants	34.5	29.4	40.0	35.2		

[a]Coastal dealers are individuals or firms buying directly from fishermen and reselling into the market system after marketing/processing services are provided.

[b]Round weight refers to the live weight basis. Round weights were used to put individual product forms such as fillets and whole products on a comparable basis. Conversion factors used were 2.63 pounds round weight to one pound fillet and 1.15 pounds round weight to one pound whole (gutted) weight.

[c]Percentages may not add to total due to rounding.

Table 2.--Percentage distribution of coastal dealer grouper sales (dollars) by product form, 1979-82 averages[a].

Product form	Total	Wholesale			Retail	
		NE	South	Total	Seafood Market	Restaurants
Total distribution:						
Fresh whole iced	63.5	13.4	44.7	58.1	2.1	3.3
Frozen whole	1.3	-	1.1	1.1	.2	-
Frozen fillets	.4	.1	.3	.4	-	*
Fresh fillets	34.7	-	3.3	3.3	.2	31.2
Distribution within South:						
Fresh whole iced	58.0		51.8		2.4	3.8
Frozen whole	1.4		1.3		.1	-
Fresh fillets	40.2		3.8		.2	36.2
Frozen fillets	.4		.4		-	*
Distribution within Northeast:						
Fresh whole iced	98.3	98.3			-	-
Frozen whole	.6	-			.6	-
Frozen fillets	1.0	1.0			-	-

[a] *represents less than .1 percent and (-) represents no reported sales in this category. Percentages may not add due to rounding.

filleted product generally demanded by the retailers and restaurants. In percentage terms 58 percent of all sales were made to wholesalers on a fresh whole iced basis. Another 31.2 percent of sales were made directly to restaurants on a fresh fillet basis. The remaining 10.6 percent of sales were made to wholesalers on a frozen whole, frozen fillet and fresh fillet basis (4.8 percent), to retail markets on a fresh whole iced, frozen whole, and fresh fillet basis (2.5 percent), and to restaurants on a fresh whole iced basis (3.3 percent).

Distribution of grouper by product form also differed by regional location of buyer which was closely related to differences in type of buyers within regions. Within the South 58 percent of grouper purchased from coastal dealers were in the fresh-whole-iced product form compared to over 98 percent in the Northeast (Table 2). Over 40 percent of the purchases within the South were fresh fillets which reflects the relatively large percentages of sales in the region going to restaurants. No sales were reported by coastal dealers to restaurants in the Northeast and less than one percent went to other retail markets.

PRICES AND MARKETING MARGINS

Survey results indicated prices received by coastal dealers tended to differ between whole (gutted) and fillet product forms but generally not between the fresh and frozen categories for each of the product forms (Table 3). During the 1979-82 period, the average price received for grouper sold whole (fresh or frozen) averaged $1.20 per pound. Fresh fillets brought $3.30 per pound while frozen fillets averaged $3.35 per pound. The price difference between these two product forms (fresh and frozen fillets) should be viewed with caution due to the small volume of product reported as frozen, unless this truly represents the proportion marketed in the frozen-fillet product form.

In order to compare price and margins for alternative product forms, prices were converted to a common weight basis. In Table 3, prices are reported on a round weight basis for purposes of comparison. On a round weight basis fresh-whole-iced and frozen whole grouper sales by coastal dealers averaged $1.04 per pound during the 1979-82 period. The difference in round weight price ($1.04) and product weight price ($1.20) represents the price necessary to account for the weight loss due to evisceration. It does not represent a return to labor, investment, profits, etc. The price of $3.30 per pound for fresh grouper fillets is equivalent to receiving $1.25 per pound of grouper in the round. The price difference between the product weight and round weight basis is larger for fillets than the whole product form because it represents a greater amount of weight loss incurred in the filleting process. The difference in round weight price for the whole-product form ($1.04) and the round weight price for the fillet product form ($1.25) represents a return to labor, investment and management for providing the additional marketing service of filleting the product. In other words, the coastal dealer averaged $.21 per pound to cover additional costs and profits associated with providing the fillet product form. This does not include the revenue to cover weight loss because this was accounted for in converting from the product weight price to the round-weight price.

The marketing margin is the difference a marketing agent and/or processor receives between the purchase price he pays and the price he receives for an equivalent unit of product. During the 1979-82 period the average exvessel price paid for whole (gutted) grouper was $.96 per pound (NMFS). On a round weight (live) basis this is equivalent to $.84 per pound. Subtracting this round weight exvessel price from the round weight prices received by coastal dealers gives the marketing margins reported in Table 3. The margin for fresh-whole-iced and frozen-whole was $.20 per pound while the margin for fillets was between $.41 and $.43 per pound. This simplified analysis assumes exvessel price does not depend on the final product form. In addition, price differences among grouper species were not considered due to unavailability on a continuous basis. If, for example, higher exvessel prices are paid for groupers to be used for filleting, the margin would

Table 3.--Prices and marketing margins by product form, 1979-82 averages.

Product form	Prices[a]		Margin[b]
	Product weight	Round weight	
	dollars per pound		
Fresh whole iced	1.20	1.04	.20
Frozen whole	1.20	1.04	.20
Fresh fillets	3.30	1.25	.41
Frozen fillets	3.35	1.27	.43

[a]Prices on a product weight basis represent price per pound as actually sold while price on a round weight basis has been converted to an equivalent price based on live weight.

[b]Marketing margin was calculated by subtracting the exvessel 1979-82 average round weight price of $.84 from round weight price received by coastal dealer.

be less. The lack of difference in margin between the fresh and frozen product form implies that the cost of freezing is equal to the cost of boxing and icing. Alternatively, higher icing costs may be offset by premium prices received for the fresh product.

Price differences (between regions) were also reviewed. The average round weight price received by coastal dealers from southern buyers (wholesale and retail) was $1.11 per pound compared to $1.05 per pound from northeastern wholesale and retail buyers. Most or all of this difference reflects the large proportion of fillets shipped to southern buyers compared to sales made to northeast buyers (Table 2). Average price received from southern wholesalers was $1.03 per pound compared to $1.05 from New York wholesalers and $1.06 from other wholesalers in the Northeast. Since all wholesalers predominantly buy the same products (fresh-whole-iced), the 2 to 3 cents per pound difference probably reflects differences in transportation costs incurred when selling to northern locations compared to southern locations. Additionally, Moore (1978) found that for local in-state sales, buyers often incurred transportation costs while coastal dealers incurred the transportation costs when sales were made to out of state buyers.

To evaluate market efficiency actual marketing/processing costs incurred should be compared with the estimated price margins. Analysis

of actual costs and returns to coastal dealers was beyond the scope of the present survey. Two earlier Florida studies (Moore, 1978 and Cato and Prochaska, 1976) suggest the margins are probably in line with actual costs incurred. Moore found the weighted average marketing costs for Florida East Coast finfish to be $17.81 per hundred pounds when shipped to New York markets. Reported costs did not include returns for equipment investment or depreciation. Cato and Prochaska found variable marketing costs to be $.18 per pound for red snapper shipped from Florida west coast locations to the New York market with an additional $.25 allocated to overhead items and profits. Neither study gave detailed consideration to multiple locations, types of buyers and price and weight losses associated with alternative product forms.

SUMMARY AND CONCLUSIONS

Grouper was found to be predominantly a southern seafood product with most production and 85 percent of sales by coastal dealers taking place with other dealers in the South. Grouper sales distribution differs from most finfish sales by coastal dealers in that over 36 percent were to retail outlets with nearly all retail sales being to restaurants located in the South. Northeast buyers were primarily wholesalers. Fresh-iced-whole grouper was the primary form of sales to wholesalers while over 90 percent of sales to restaurants were in the form of fresh fillets.

Prices and marketing margins differ between whole product forms and fillet product forms but not between fresh and frozen products. The marketing margin ranges from $.20 per pound for fresh-whole-iced grouper to $.41 per pound for fresh fillets. Most of the difference between prices paid by southern buyers and those paid by northeastern buyers reflects the large proportion of fillet sales made to southern restaurants. For the same product form the price difference of 2 to 3 cents per pound between southern and northeastern buyers may reflect differences in transportation costs between the regions.

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Plesiomonas shigelloides: A NEW PROBLEM FOR THE OYSTER INDUSTRY?

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INTRODUCTION

Plesiomonas shigelloides has for a number of years been suspect regarding its ability to cause gastroenteritis in humans. Recently, attention has again focused on this organism as the possible causative agent in several outbreaks of gastroenteritis (2,9,11,14), the most recent one occurring in Florida in April of 1983 (personal communication). This report listed 29 total cases that occurred over a six day period, all associated with the consumption of raw oysters. The illness was characterized by diarrhea (100%), nausea (70%), abdominal cramps (68%), vomiting (56%), fever (44%) and chills (32%). In addition, blood was noted in the vomitous of two patients and in the stool of one patient.

Plesiomonas shigelloides, originally described by Ferguson and Henderson (4), has been variously named Paracolon C27, Pseudomonas shigelloides, Pseudomonas michigani, Fergusonia shigelloides and Aeromonas shigelloides. Based on both biochemical and morphological characteristics, it was transferred from the genus Aeromonas to Plesiomonas in 1962 (6). Plesiomonas is in the Family Vibrionaceae which also includes Aeromonas and Vibrio.

The organism is a motile gram negative rod, oxidase and catalase positive, ferments various carbohydrates with the production of acid but no gas and is sensitive to the vibriostatic agent 0/129. If an oxidase test is not conducted, it can easily be misidentified as a member of the Enterobacteriaceae, which are oxidase negative. P. shigelloides is also differentiated from the Enterobacteriaceae by its polar flagella; the Enterobacteriaceae have peritrichous flagella. It is easily differentiated from the genus Aeromonas by its ability to ferment inositol and its lack of exoenzyme production.

Plesiomonas shigelloides has been isolated from a number of mammals, birds and fish as well as water. Van Damme and Vandepitte (15) hypothesized that the high incidence of this organism in fish (59%) was a possible source of sporatic diarrhea in the tropics. Rutala et al. (11) was able to isolate this organism from 1 of 10 oysters harvested from the same area as the oysters involved in an outbreak; whereas, Arai et al. (1) isolated this organism from 12.8% of 497 river water samples and only 0.0078% of 38,454 healthy Tokyoites.

Isolation of Plesiomonas shigelloides has usually been accomplished on enteric agars during the routine analysis of stool samples as its growth characteristics are not unlike those of the Enterobacteriaceae. Hu (7), during a survey of the Suwannee River estuary was unable to recover P. shigelloides from any samples. He attributed this failure to a lack of a suitable selective and differential medium for its isolation.

In an attempt to better understand this organism, a study was undertaken of isolation methods, identification procedures, growth characteristics and its distribution in the estuarine environment, particularly oysters.

MATERIALS AND METHODS

Environmental and marine animal samples were obtained from the area of Suwannee, FL in May 1983 for a brief survey of the incidence of Plesiomonas shigelloides. All samples were blended at 8000 RPM in a Hamilton Beach or Waring blender using Butterfield's Phosphate buffer to prepare the dilutions. A 0.1 ml aliquot was surface inoculated onto duplicate Plesiomonas Agar plates. This medium, formulated in our laboratory, contains in g/l: 1.0 peptone, 5.0 NaCl, 2.0 yeast extract, 7.5 mannitol, 5.0 arabinose, 1.0 inositol, 2.0 lysine, 1.0 bile salts No. 3, 0.08 phenol red, and 15.0 agar. The pH is adjusted to 7.4 before adding the agar. Typical colonies are 1-2 mm in diameter, pink and cloudy. Following incubation at 35C, colonies were transferred to both Triple Sugar Iron (TSI) agar slants and Inositol Gelatin media. Typical Plesiomonas isolates have an alkaline slant with an acid butt in TSI. They ferment inositol with the production of acid but no gas and do not liquify gelatin. A test for oxidase was performed from the TSI slants; Plesiomonas is oxidase positive. Isolates giving typical reactions at this time can be considered to be Plesiomonas shigelloides. All media used in this study were Difco products, Difco Laboratories, Detroit, MI.

A brief media and temperature of incubation study was also conducted. Several different modifications of the original Plesiomonas Agar were evaluated at 35 and 42C. It was thought that 42C would eliminate some of the competing microflora but still allow Plesiomonas to grow.

In the initial study water samples from lakes, creeks and ponds in the Gainesville, FL area were used. Five media were tested. Media 1 was the original Plesiomonas Agar. Media 2 was the original media with inositol and peptone increased to 0.3 and 0.5% respectively. Media 3 was the original media with 2% gelatin added. Media 4 was the original media with increased inositol (0.3%) and peptone (0.5%) plus 2% gelatin. Media 5 was a modified Inositol Gelatin agar, which contained in g/l: 5.0 peptone, 5.0 NaCl, 2.0 yeast extract, 5.0 inositol, 1.0 bile salts no. 3, 0.08 phenol red, 20.0 gelatin, and 15.0 agar. The pH was adjusted to 7.4 before adding the agar. Plesiomonas colonies are yellow with no zone of gelatin hydrolysis on this media.

Typical colonies were identified as before and the media were compared to determine which yielded the best recovery rate with the least false colonies picked.

Based on the results of the initial study, another experiment was conducted to compare Media 1 and 4 at 35 and 42C using water from the Gainesville, FL area and water, sediment, weeds and oyster samples from the Suwannee, FL area.

RESULTS AND DISCUSSION

The data would indicate that P. shigelloides is rather widespread in the estuarine environment (Table 1). Based on a limited sampling regime, P. shigelloides was isolated from bream, mullet, crabs, grunt, marsh clams, water and 11 of 40 oyster samples. Ten of the eleven positive oyster samples

Table 1. List of Suwannee River samples either positive or negative for the isolation of Plesiomonas.

Positive		Negative	
Sample	Number	Sample	Number
Bream	7	Crab	1
Crab	2	Crawfish	1
Grunt	1	Eel	1
Marsh Clam	3	Hydrilla	3
Mullet	1	Mullet	1
Oysters	11	Oysters	29
Water (estuarine)	4	Sediment	1
		Trout	1
		Water (estuarine)	2

were shellstock, and one was a pint sample of retail oysters. Based on visual observation of the isolation plates, the number of cells present was esti_ mated to be low, probably less than 100 organisms per gram. However, levels of P. shigelloides in the bream samples were much higher and may reflect a host relationship with this species of fish. Other workers have noted that fish may be contaminated with P. shigelloides at levels from 10 to 60% of the samples analyzed(1,15); however, no indication of the numbers of organisms present was mentioned.

The initial experiment involving 5 different plating media and 2 temperatures determined that Media 1 and 4 were the most effective, and it seemed that 42C did decrease the level of competing microorganisms without adversely effecting the recovery of the Plesiomonas present (results not shown). These two plating media are very similar and rely on the absence of fermentation of the major carbohydrates present followed by a positive lysine reaction to give a pink or red zone around the Plesiomones colonies. Even in Media 4 where the amount of inositol is increased yielding a pale yellowish colony, there is still a faint pink zone around the Plesiomonas colony.

Based on the above results, a second experiment involving Media 1 and 4 with incubation at 35 and 42C was conducted. The results (Table 2) show that Media 1, the original media developed in our laboratory, was best for the recovery of P. shigelloides. The use of 42C did seem to be more effective than 35C, but it should be noted that in 5 of the 15 samples, Plesiomonas was recovered at 35C but not at 42C in Media 1. In only 2 samples was Plesiomonas recovered at 42C but not at 35C in Media 1. This could be due to over selectivity at 42C, especially if the organisms were injured. In many Suwannee River samples where the level of Plesiomonas seemed to be low, the 42C plates yielded no colonies. The Gainesville area water samples had a much higher level of contamination of all kinds including Plesiomonas, and the 42C plates had abundant growth. The Gainesville area water samples came from shallow

Table 2. Effect of medium and incubation temperature on the recovery of P. shigelloides.

Samples	Media 1		Media 4	
	35C	42C	35C	42C
Gainesville pond water	1/2[a]	2/2	0/2	3/3
Gainesville pond water	3/3	4/4	2/3	3/4
Gainesville creek water	3/3	3/3	1/3	3/4
Gainesville lake water	1/1	1/2	2/2	4/4
Suwannee water	0/2	1/1	0/1	0/0
Suwannee water	4/4	0/0	1/1	0/1
Suwannee water	1/1	0/0	1/1	0/2
Suwannee sediment	3/3	0/0	0/1	2/4
Suwannee weeds	0/1	3/3	0/1	1/6
Suwannee River oysters	0/0	0/0	0/0	0/1
Suwannee River oysters	1/1	0/0	0/2	0/0
Suwannee River oysters	1/1	0/0	0/2	0/2
Suwannee River oysters	0/2	0/0	0/1	0/2
Suwannee River oyster	2/2	1/1	1/4	2/3
Suwannee River oysters	0/1	0/1	0/1	1/2
Total	20/27	15/16	8/25	20/38
% Positive	74	94	32	53

[a]Number isolates Plesiomonas/Number of isolates picked.

creeks, ponds and small lakes which are located in highly developed areas, whereas the Suwannee River runs deeper and has much less development. More extensive experimentation needs to be done to determine the ideal conditions for isolation of Plesiomonas shigelloides from water and oyster samples.

A major problem associated with this organism has been the inability of various workers to demonstrate pathogenicity under laboratory conditions. Most associations with disease have been epidemiological in nature. Various systems have been employed in attempts to elicit a positive pathogenic response including ligated ileal loops, Serenyi test, Y-1 adrenal cells as well as suckling mice, with limited success (5,8,10,12,13). However, the epidemiological data is rather strong linking this organism with gastroentestinal disorders (2,3,9,11,14). These reports have included water as a major problem as well as raw oysters.

Little is known regarding the conditions necessary for growth of P. shigelloides in foods or the levels of the organism necessary for infection. Until many of these and other factors are understood, technologist should be aware that P. shigelloides represents a potential problem to the safe consumption of raw oysters.

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REPACKAGING OF FRESH OYSTERS, CLAMS AND MUSSELS

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There are two types of repackaging of fresh oysters, clams and mussels that are of concern to public health regulatory agencies. The first is repackaging of these products at any point after the initial packaging, even when accomplished by certified processors meeting all registration and labelling requirements. The second type is becoming widespread even though it is illegal in all states. That is the practice of on premise repackaging of these products for display and sale at retail establishments. The problem with both types is similar, though more specific with the on premise repacking. The repacking results in loss of the public health safe identity of these shellfish.

All shellfish producing states and several foreign countries operating under Memorandums of Understanding with the U.S. Food and Drug Administration (FDA) classify estuarine waters according to suitability for harvesting safe, wholesome oysters, clams and mussels. These Programs also inspect and certify packers and shippers in compliance with standards set forth under the National Shellfish Sanitation Program (NSSP). Container labelling and records maintenance is required to reflect the name, address, and valid certificate number of the packer/shipper and other information pertinent to source identification in the event that a disease outbreak is attributed to consumption of these shellfish. FDA reviews state programs and those of participating nations to determine compliance with standards also established in the NSSP and publishes a monthly listing of firms currently certified to process, package, and ship these shellfish.

Oysters, clams and mussels are filter feeders, pumping large quantities of water through their systems and concentrating in their bodies any nutrients and/or pollutants which may be present. Being essentially immobile creatures, they are, so to speak, victims of their environment. These animals are commonly eaten raw, gut and all. They are capable of carrying dangerous concentrations of water borne disease organisms, such as cholera, diphtheria, and other gastroenteric disorders. Public health authorities, therefore, feel that an effective shellfish control program is imperative.

Shellfish which have been processed and packaged outside the various Programs or which have been removed from containers with the required labelling, cannot be identified as to source or handling practices and therefore are suspect with regard to consumer safety. Obviously then, any product repackaged on premises by an uncertified establishment has lost its identity and should not be consumed. There is no assurance that mishandled shellfish or shellfish from improper sources, are not being offered for sale. Shellfish in containers bearing the proper labelling, including a current certification number, provide the assurances of having been processed, packaged and handled under the auspices of a uniform state/national/foreign program which establishes standards for these practices.

This returns us to the first type of repackaging, that accomplished by certified processors who place the repackaged shellfish into containers bearing legitimate labelling including a certificate number. The assurances of being processed and packaged under one of the Programs remains, but there are questions arising as to loss of source identity when products from different lots, states, or bay systems may be mixed to be repacked. These questions and many others concerning the safety of shellfish are arising as a result of changes in consumer habits, the economy, transportation and refrigeration improvements, and many other factors affecting this industry. This particular question is currently under consideration by the Interstate Shellfish Sanitation Conference (ISSC)

The ISSC is a formal organization of state shellfish regulatory authorities, shellfish industry representatives, and FDA representatives, which has adopted the NSSP Manual as its basic program. The ISSC has also established procedures to maintain, update, and revise when necessary any portion of the program or procedures in order to continue to provide consumer protection. This program, adopted as the Interstate Shellfish Sanitation Program (ISSP), serves as the recommended guidelines for sanitary control of shellfish in this country. The Conference and the ISSP provide not only a program, as the NSSP did, but a formal, continuous, tri-partite organization, meeting annually to maintain the program and respond to changing situations in today's changing world.

Hopefully the Packaging and Labelling Committee of the ISSC can recommend a solution to questions concerning repackaging within the program that the Conference can adopt and recommend as the uniform nationwide solution to this problem.